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RESEARCH PAPER

Chlorthalidone inhibits the KvLQT1 potassium current in guinea-pig ventricular myocytes and oocytes from *Xenopus laevis*

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Background and purpose: Chlorthalidone is used for the treatment of hypertension as it produces a lengthening of the cardiac action potential. However, there is no experimental evidence that chlorthalidone has electrophysiological effects on the potassium currents involved in cardiac repolarization.
Experimental approach: Ventricular myocytes and oocytes, transfected with human ionic channels that produce *I*K current, were exposed to different concentrations of chlorthalidone. Action potentials and potassium currents were recorded using a patch clamp technique. To determine which component of the current was affected by chlorthalidone, human channel proteins (hERG, minK and KvLQT1) were used.
Key results: Chlorthalidone prolonged the ventricular action potential at 50 and 90% by 13 and 14%, respectively. The cardiac potassium currents I_{to} and IK_1 were not affected by chlorthalidone at any concentration, whereas the delayed rectifier potassium current, *I*K, was blocked in a dose-response, voltage-independent fashion. In our preparation, 100 μM chlorthalidone blocked the two components of the delayed rectifier potassium current with the same potency ($50.1 \pm 5\%$ for IK_r and $54.6 \pm 6\%$ for IK_s) ($n = 7$, $P < 0.05$). The chlorthalidone-sensitive current was slow and saturated at potentials greater than $+30$ mV. In our conditions only the KvLQT1 potassium current was affected by the drug, by 14%.
Conclusions and implications: Chlorthalidone was demonstrated to have a direct effect on cardiac ventricular myocytes; it blocked the delayed rectifier potassium current (*I*K), specifically the KvLQT1 component of the potassium current. These results indicate that it has potential for use as an antiarrhythmic but further studies are needed.
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Keywords: chlorthalidone; thiazide diuretics; cardiac potassium currents; *I*K; patch clamp; isolated cardiac myocytes; KvLQT1 current; oocytes

Abbreviations: I_{to}, transient outward potassium current; *I*K, delayed rectifier potassium current; IK_r, fast component of *I*K; IK_s, slow component of *I*K; IK_1, inward rectifier potassium current; hERG, human ether a go-go channel; minK, minimum potassium channel; KvLQT1, potassium long Q-T channel

Introduction

Several types of potassium currents are involved in the repolarization of the cardiac action potential. Variations in the type and relative sizes of these potassium currents throughout the heart generate various action potential waveforms. In the ventricles, the main outward potassium currents are the transient outward potassium current (I_{to}); the delayed rectifier potassium current (*I*K), with its two components, fast (IK_r) and slow (IK_s); and the inward rectifier potassium current (IK_1) (Armstrong and Hille, 1998; Volders *et al.*, 1999; Hille, 2001).

These currents participate actively during cardiac repolarization: I_{to} activates at the end of the upstroke of the action potential, and it is responsible for the early repolarization period (Josephson *et al.*, 1984). The two components of *I*K are turned on during phases 2 and 3 of the action potential and partially determine the duration of the plateau phase. IK_1, meanwhile, has an essential role in the last stages of the repolarization process (Sanguinetti and Jurkiewicz, 1990; Hille, 2001).

The prolongation of the ventricular action potential produced by some antiarrhythmic drugs leads to a lengthening

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of the refractory period, and this is considered to have an antiarrhythmic effect (Sanguinetti and Jurkiewicz, 1990; Carmeliet and Mubagwa, 1998). So, manipulation of the potassium currents may lead to an increased knowledge as to how arrhythmic events take place, and how they may be terminated. The effects of several potassium-blocking drugs like sotalol (Berger *et al*., 1989), dofetilide (Weerapura *et al*., 2002) and E-4031 (Lynch *et al*., 1995) have been investigated and, more recently, the activities of a set of drugs with possible potassium-blocking effects like chloroquine (Sanchez-Chapula *et al*., 2001), indapamide (Fiset *et al*., 1997) and azimilide (Xue *et al*., 1999) are being studied.

Recently, attention has focused on a family of drugs known generically as thiazide diuretics, which appear to be able to block potassium channels. Several drugs trials have indicated that these diuretics, in combination with ACE inhibitors, calcium antagonists or β-receptor blockers, may be appropriate antihypertensive treatments for some patients (Lawrence, 2002). However, the trials seem not to indicate the advantage of using one drug alone or a combination of them. Apart from the benefit obtained from a reduction of blood pressure, it is not known, if the effects of these thiazide diuretics on the heart are due to their direct action on cardiac tissues and, specifically, on potassium currents.

Diuretics like chlorthalidone frequently cause modest hypokalaemia, and it is probable that this electrolyte disturbance may contribute to its effect on cardiac tissues. Since chlorthalidone is a sulphonamide derivative, it is likely that it has direct actions on cardiac potassium currents. Thus, the aim of this study was to determine the actions of the thiazide diuretic chlorthalidone on the inward and outward potassium currents in guinea-pig ventricular cardiac myocytes, and investigate its effects on the different components of the human *IK*, human ether a go-go channel (hERG), minimum potassium channel (minK) and potassium long Q-T channel (KvLQT1) transfected in oocytes from *Xenopus laevis* frogs.

Methods

Isolation of myocytes

Guinea-pig isolated cardiac myocytes were prepared according to a standard enzymatic perfusion method (Clark *et al*., 1993; Bouchard and Fedida, 1995). Briefly, adult guinea-pigs (200–250 g) were given an i.p. dose of heparin (1000 U kg^{-1}) and anaesthetized with sodium pentobarbital (35 mg kg^{-1}, i.p.). The hearts were excised and perfused for 5 min through the aorta using a Langendorff system at 37 °C, with normal Tyrode solution of the following composition (in mM): 140 NaCl, 4.5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose and 5 Na-pyruvate at pH 7.4. Thereafter, the hearts were perfused with a nominally zero-calcium Tyrode solution for a further 5 min. The solution was then changed to a nominally zero-calcium solution containing collagenase (Type 1A 0.02 mg ml^{-1}) and protease (Type XIV 0.01 mg ml^{-1}) for approximately 5 min. The enzymes were washed out by perfusion with KB (Kraft–Brühe) (Isenberg and Klockner, 1982) solution for 5 min. Small pieces of ventricular free wall (1 mm^3) from both the left and right ventricles were dissected and placed into separate flasks containing the storage solution. Single cells were obtained by mechanical agitation with a pipette and stored in KB solution with no Ca^{2+} at low temperature (4 °C). During the electrophysiological experiments, the cells were superfused with normal Tyrode solution, at a perfusion rate of 1 ml min^{-1}. In all cases, the perforated patch-clamp recordings were conducted at 35–37 °C.

Preparation of oocytes and microinjections

Adult *Xenopus laevis* female frogs (Xenopus I, Ann Arbor, MI, USA) were anaesthetized by immersion in 0.2% MS-222. Stage V and VI oocytes were surgically removed, placed in OR-2 buffer containing (in mM) 82.5 NaCl, 2.5 KCl, 1 $MgCl_2$ and 5 HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid) at pH 7.6, and treated with collagenase (Type 1A, 1.3 mg ml^{-1}) to remove the follicular membrane. The nuclei of the oocytes were injected by using a nanolitre automatic injector with 2–10 ng of hERG, minK and KvLQT, α-subunit. Eggs were then maintained at 18 °C in ND-96 solution (in mM, 96 NaCl, 2 KCl, 1 $MgCl_2$, 5 HEPES and 1 $CaCl_2$ at pH 7.6), supplemented with 0.5 mM theophylline, 0.5 mM pyruvate and 50 μg ml^{-1} gentamicin for up to 3 days before recording.

Electrophysiological recording in oocytes

Oocytes were placed in a 1.6-ml recording chamber and continuously superfused with a chloride-low solution using MES (2-(*N*-morpholino) ethanesulphonic acid hydrate; 4-morpholine-ethanesulphonic acid; Kamiya *et al*., 2006), at a flow rate of approximately 1 ml min^{-1}. Two electrode voltage-clamp recordings were made at room temperature (20–22 °C) using an OC-725C amplifier. Electrodes were pulled on a horizontal puller (P-97). Agarose-cushion electrodes filled with 3 M KCl (Schreibmayer *et al*., 1994) were used to achieve a final resistance of 0.6–1.2 MΩ. Potassium current signals were digitized at a sampling rate of 10 kHz by an analogue-to-digital converter and stored on a computer for analysis with pClamp software (Version 8.02; Axon Instruments, Foster City, CA, USA). Potassium currents (*IK*) were elicited by step depolarizations from a holding potential of −60 mV at 0.1 Hz unless otherwise indicated. The amplitude of expressed *IK* was typically 1–7 μA. Only oocytes with *IK* peak lower than 7 μA were used in the present study, to minimize voltage-clamp errors (Li *et al*., 1999; Orta-Salazar *et al*., 2002). Current–voltage (*I–V*) relationships were determined from peak currents elicited by 6- to 8-s, 10-mV steps from a holding potential of −60 to +60 mV. The voltage dependence of steady-state inactivation of K^+ channels was determined by a two-pulse protocol. A first variable voltage conditioning pulse that lasted 4 s was applied to inactivate different fractions of the potassium channels. After 2 ms, a second 2-s long test pulse was applied at +50 mV. Data were normalized to the maximum potassium current recorded during the test pulse.

Solutions for myocytes

The composition of the external solution for recording I_{to} and IK_1 potassium current was (mM) 120 NaCl, 5.4 KCl, 10 Hepes-Na, 1.8 $CaCl_2$, 1$MgCl_2$, 5 glucose and 2 $CoCl_2$ at pH

7.4 with NaOH 1 M. The composition of the external solution for recording *I*K current was (mM) 136 NaCl, 4 KCl, 1 $CoCl_2$,1 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes and 11 glucose at pH adjusted to 7.4 with NaOH.

The composition of the internal solution was (mM) 80 K-aspartate, 10 KH_2PO_4, 50 KCl, 1 $MgS0_4$, 5 Hepes and EGTA 5 at pH adjusted to 7.3 with KOH. In all cases, we used 100 μM amphotericin B to develop the perforated patch-clamp technique.

Chlorthalidone stock solution (10 mM) was prepared in 0.2 M KOH, and aliquots were added to the extracellular solution to achieve the desired final drug concentrations (3–300 μM). The total K^+ ionic concentration was adjusted to 5.4 mM.

Solutions for oocytes

The composition of the external solution for recording *I*K and KvLQT1 potassium current was (mM) 96 Na-Mes, 2 K-Mes, 2 Ca-Mes, 1 $MgCl_2$ and 5 Hepes at pH 7.6 with methane-sulphonic acid. Microelectrodes were pulled from borosilicate glass capillary tubes to obtain a final resistance of 0.5–1.0 MΩ when filled with 3 M KCl.

Electrophysiological recording of single myocytes

Isolated single cells were placed in a small-volume (0.2 ml) recording chamber on the stage of an inverted microscope (IM35). Macroscopic current recordings under voltage-clamp conditions were obtained using the perforated whole-cell recording method (Hamill *et al*., 1981) and an Axopatch 1D amplifier. Glass pipettes were pulled from borosilicate capillary tubing (TW 150) using a horizontal puller (P-97). Membrane potentials were corrected for the liquid junction potential (~6.8 mV). Currents were filtered with an eight-pole Bessel filter at 2 kHz, digitized at 5 kHz and stored on a computer hard drive. In a typical voltage-clamp experiment, the time required for the membrane potential to reach 95% of its final value following a step in command potential is equivalent to the product of three times the membrane time constant (τ_m). Prior to the point at which the membrane capacitance is fully charged, membrane currents measured by a voltage-clamp amplifier will partly be due to charging of the membrane capacitance and partly due to the flow of ions through channels. Indeed, in previous studies, it has been shown that serious voltage-clamp errors can occur, both at 18 and 37 °C, when large currents, like *I*K, are not adequately controlled. Such errors can lead to incorrect measurements of the voltage dependence of *I*K. Therefore, careful control of the membrane potential is needed during the voltage clamping of *I*K. In the present study, the seal resistance was usually between 2 and 5 GΩ, and the series resistance was compensated between 80 and 90% in each cell to provide for optimal voltage-clamp integrity. In a total of seven myocytes, the average (± s.e.mean) DC pipette resistance was 3.26 ± 0.2 MΩ, the compensated series resistance was 1.85 ± 0.1 MΩ and cell capacitance was 92 ± 12 pF, yielding an average τ_m of 158 μs.

Statistics and data analysis

Results are expressed as the mean ± s.e.mean. Differences between mean data were analysed with a paired or unpaired Student's *t*-test as appropriate. All the currents were analysed using the pClamp version 8.02 software (Axon Instruments). *I*K currents were elicited by depolarizing pulses (duration, 3 s, unless otherwise stated; frequency, 0.1 s, 10 mV steps from −40 to +70 mV). The tail envelopes were elicited by a fix-voltage pulse to +50 with variable duration from 1 ms to 3.2 s. The relationship between the *I*K total amplitude and the tail amplitude was the degree of activation of the two components of the *I*K potassium current. The average data were later best fit by a double exponential of the formula: $f(y) = A\exp(-t/\tau_1) + B\exp(-t/\tau_2) + D$, where A and B are amplitude terms, t is time; τ_1, τ_2 are time constants for the fast and slow inactivation phases and D is the amplitude of the steady-state component. Activation and inactivation plots were generated by dividing the $I\mathrm{K}_{peak}$ (total amplitude at the end of the voltage pulse), measured at a given potential by the difference between measured and reversal potential. Average data were best fitted with a Boltzmann distribution equation: $G/G_{max} = 1/1 + \exp((V_{1/2} - V)k)$, where G is the conductance, G_{max} the maximum conductance, $V_{1/2}$ is the potential at which half the channels are activated and k the slope factor. For the inactivation plots: $y = 1/\{1 + \exp(V - V_{m1/2})/k\} + A$, where y is normalized *I*K, A the baseline, V_m the membrane potential, $V_{m1/2}$ the voltage of half-maximal inactivation and k a slope factor.

Action potentials were recorded in the whole-cell current-clamp mode and were elicited by the injection of depolarizing pulses (duration, 3 ms; amplitude, 1 nA; frequency, 0.1 Hz at 37 ± 1 °C).

The dose–response curve for chlorthalidone effects was adjusted by the WinNonlin program (Pharsight Co., version 2.1) to calculate its IC_{50} value and 95% confidence limits according to the following model: $I\mathrm{K} = 1/(1 + ([\text{chlorthalidone}]/IC_{50})^n)$. A P-value <0.05 was used to denote significant differences between groups.

Sources of materials

Collagenase (Type 1A), protease, MS-222 (Type XIV), amphotericin B and chlorthalidone were all obtained from Sigma-Aldrich (Quimica, Mexico, D.F.). KvLQT, α-subunit, was kindly provided by Dr M Sanguinetti (University of Utah). The nanolitre automatic injector (model A203XVY) was from World Precision Instruments (WPI) (Sarasota, FL, USA); the OC-725C amplifier from Warner (New Haven, CT, USA,); the analogue-to-digital converter from Digidata 1200 (Axon Instruments); the horizontal puller (P-97) from Sutter Instruments (Novato, CA, USA); the inverted microscope (IM35) from Zeiss (Heidelberg, Germany); the Axopatch 1D amplifier from Axon Instruments and the borosilicate capillary tubing from TW 150 (World Precision Instruments).

Results

Effects of chlorthalidone on the action potential

Chlorthalidone has a marked effect on the electrical activity of the cardiac myocytes. Figure 1 shows this effect under several conditions: control, superfusion of 10, 30 and 100 μM chlorthalidone and after washout of the drug. The duration

of the action potential at 50 and 90% of total repolarization was increased by 2, 21 and 35%, and 3, 19 and 37%, respectively, without a noticeable change in the resting potential, phase 0 of the action potential or overshoot. The effect of the drug was partially reversed after a 15-min washout.

The potassium currents involved in the repolarization process have been studied for some time, as mentioned previously. We decided to study those currents one at a time to obtain more information about the effect of chlorthalidone on the duration of the action potential.

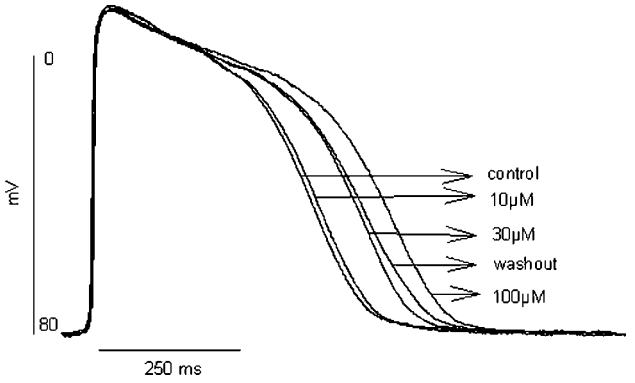

Figure 1 Effect of chlorthalidone on the action potential of guinea-pig left ventricular myocytes (basic cycle length (BCL) = 1000 ms, temperature 36.5 °C). The recordings were obtained under basal conditions (control), after a perfusion of 10, 30 and 100 μM chlorthalidone, and following a 15-min washout. The duration of the action potential was increased without an effect on the total amplitude or the resting membrane potential.

To determine if the drug had any effect on the I_{to} or IK_1 potassium currents, experiments were performed in a set of myocytes superfused with a 100-μM chlorthalidone. Figure 2 illustrates the current records in two different myocytes obtained from the left ventricular wall. Figure 2a shows I_{to} in basal conditions and after the perfusion of the drug. Figure 2b shows the original trace in another myocyte, in which we investigated the IK_1 potassium current under the same conditions as those in Figure 2a. No effects were recorded in either group.

On the basis of the fact that the I*K* current is at least in part responsible for the repolarization process, we decided to investigate this current using the 'perforated' whole-cell patch-clamp technique (to prevent the rundown of the current) in ventricular cardiac myocytes. Figure 3a shows the currents elicited in a myocyte exposed to the same chlorthalidone concentration as in Figure 2. Superfusion of 100 μM chlorthalidone decreased the *IK* by 50 ± 5% after applying a +70-mV depolarizing pulse. When the physiological step voltage between 0 and +20 mV (overshoot of the action potential in cardiac cells) was applied, the drug produced a similar current decrease of 52 ± 4 and 55 ± 7%, respectively. Figure 3b shows the current–voltage relationship for a variable drug concentration: 3, 5, 10, 30, 100 and 300 μM for the total amplitude of the *IK* current and of the *IK* tail currents. Chlorthalidone produced a concentration-dependent inhibition of both components, and it seems

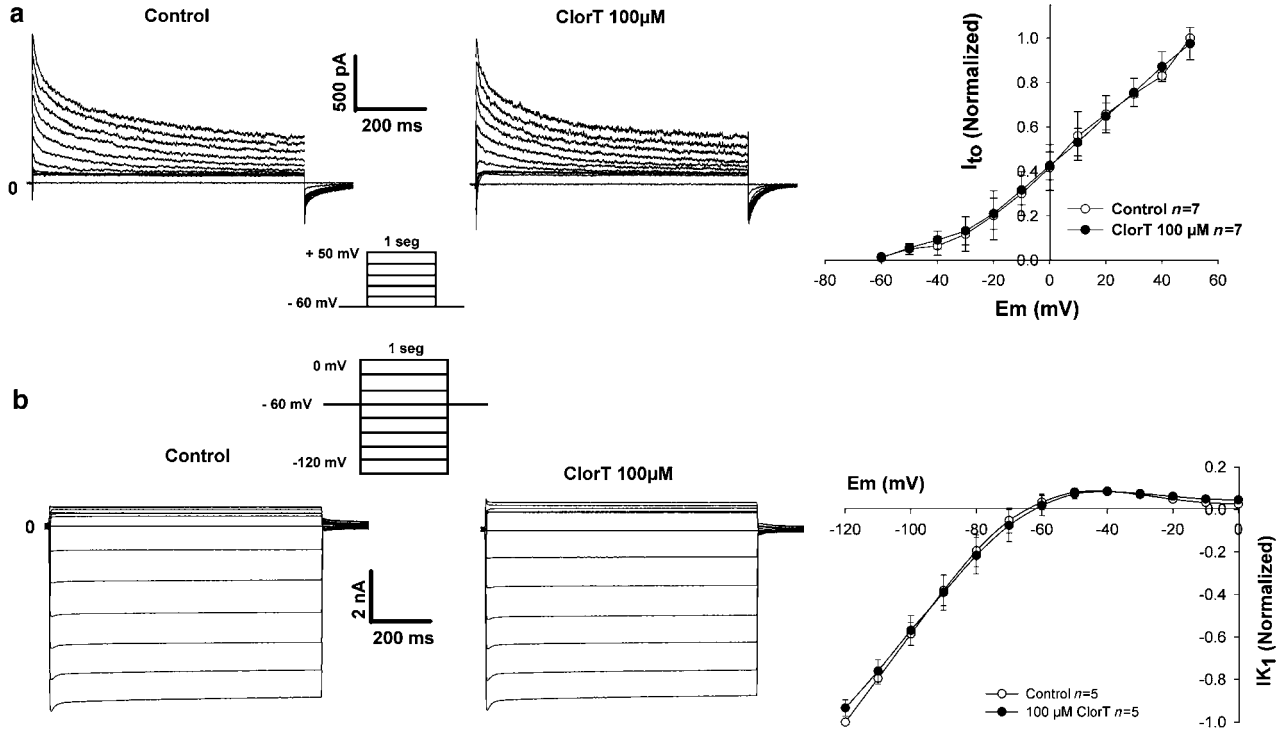

Figure 2 Effect of chlorthalidone on inward and outward potassium currents recorded from left ventricular myocytes. In (**a**), depolarizing pulses from a holding potential of −60 to +50 mV produced a fast inactivating outward potassium current, followed by a slowly activating outward potassium current. Chlorthalidone did not significantly affect either component of the transient outward potassium current (I_{to}; right, $n = 7$). (**b**) Depolarizing pulses from −120 up to 0 mV produced an inward potassium current in control conditions, and after the addition of chlorthalidone ($n = 5$). No significant changes were observed.

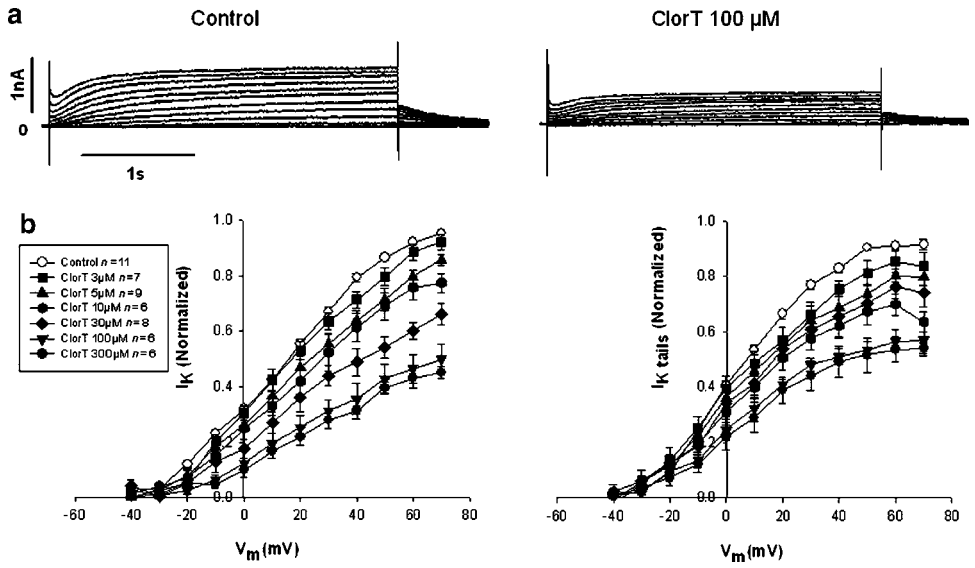

Figure 3 Blocking effect of chlorthalidone on the outward potassium current *I*K in left ventricular myocytes. (**a**) Currents elicited by long depolarizing pulses up to +70 mV from a holding potential of −40 mV under control conditions and after the addition of chlorthalidone. (**b**) Total and tail current–voltage relationships of the delayed rectifying potassium current (*I*K) in the presence of several chlorthalidone concentrations.

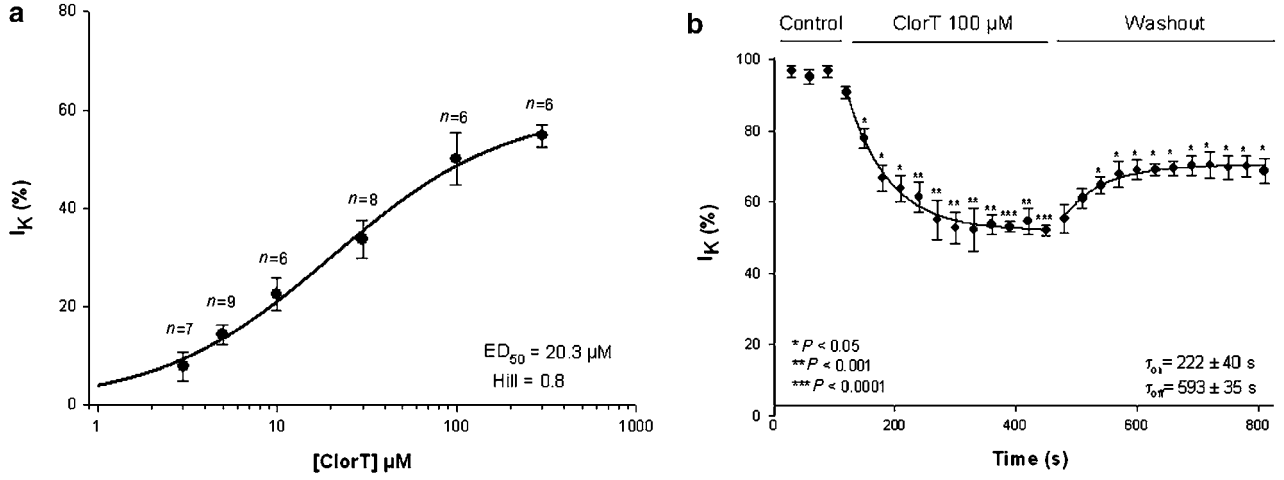

Figure 4 Dose–response relationship and time course for the effect of chlorthalidone. (**a**) Concentration dependence of blocking effect of chlorthalidone on the total amplitude of the delayed rectifying potassium current (*I*K), measured at the end of each voltage step. Each point represents the average of six–nine experiments ± s.e.mean. The line represents the best fit obtained using the Hill equation (see Methods). (**b**) Time course of the chlorthalidone effect. The first 3 points correspond to control conditions, followed by perfusion of 100 μM chlorthalidone for 8 min, and after a 5-min washout. Data obtained between 180 and 460 s were fitted using a simple decay exponential equation. Mean data between 500 and 830 s were fitted using a simple raise exponential model. Each point represents the mean ± s.e.mean of six experiments.

that the maximum effect was reached with 100 μM chlorthalidone.

To summarize the effect of chlorthalidone on the *I*K current, we plotted the inhibitory effect for several drug concentrations. The average data were best fitted using a Hill equation (see Methods). The IC_{50} for the inhibitory effect of chlorthalidone on the *I*K current was estimated to be 20.3 μM with a Hill number of 0.8 (Figure 4a). Thus, in the following experiments, we used 100 μM chlorthalidone to ensure blockage of the drug-sensitive component of the *I*K current. Figure 4b shows the average data for the temporal course of the blocking effect of chlorthalidone. The external application of 100 μM chlorthalidone progressively blocks the current (at +70 mV, 50 ± 3%) with a τ_{on} of 222 ± 40 s; the effect was partially reversible after an ~5-min washout, with a τ_{off} of 593 ± 35 s.

Figure 5 shows the chlorthalidone-sensitive currents that were obtained by digital subtraction of currents measured in basal conditions, and after the addition of 100 μM chlorthalidone. Figure 5a shows the measurement of both components of the outward current on a myocyte before and after the addition of chlorthalidone. The lower record shows the digital subtraction of the currents. The mean current–voltage relationship plots for both time-dependent currents measured during step depolarization (Figure 5b) and amplitudes of the tail currents measured upon repolarization to −40 mV

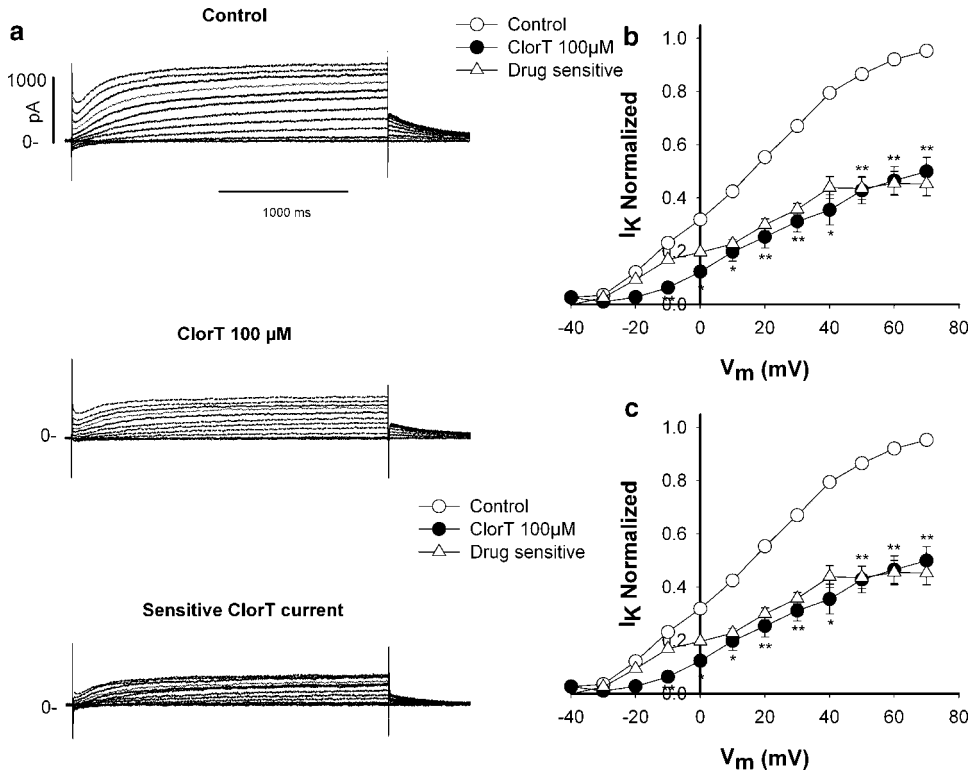

Figure 5 Chlorthalidone-sensitive rectifying potassium current. (**a**) Original recordings of delayed rectifying potassium current (*I*K) from left ventricular myocytes. The currents were elicited by long depolarizing pulses from a holding potential of −40 up to +70 mV, in control conditions (top), and after the addition of 100 μM chlorthalidone (middle). The chlorthalidone-sensitive current (bottom) was obtained by digital subtraction of the two previous currents. In (**b**), the current–voltage relationship for 6–11 cells is shown. The total amplitude was measured at the end of the voltage pulses, in basal conditions and after the perfusion of 100 μM chlorthalidone. Note that the chlorthalidone-sensitive current saturated at potentials positive to +40 mV. (**c**) Plot of normalized tail current measurements at its maximum amplitude just after repolarization. The amplitude of the control currents was taken as 1. Again, the chlorthalidone-sensitive tail current was saturated at potentials positive to +40 mV.

(Figure 5c) are also shown. In each graph, the amplitudes of control currents, currents measured after the exposure to 100 μM chlorthalidone and drug-sensitive current are plotted. The drug has a strong effect on both currents (the total amplitude and the tail current) without an apparent change in morphology or in the onset of activation. The drug-sensitive current has no effect on the activation time, and it is fully activated during these pulses at potentials greater than +20 mV (Figure 6a). At test potentials greater than −20 mV, the currents decrease during depolarizing pulses, whereas the tail currents saturate at potentials over +30 mV. This suggests that the drug blocks the inward rectification of the current, or that it may have a strong voltage-dependent effect.

To determine whether the effect of chlorthalidone is voltage-dependent, we tested the effect of several drug concentrations on the total current–voltage relationship of the I*K* current. Mean data of the current inhibition show that the blocking effect of chlorthalidone on the *I*K current is not voltage-dependent (Figure 6b).

Finally, the effect of chlorthalidone on the *I*K current may explain its ability to lengthen the action potential (see Figure 1), but it remains to be determined whether the drug acts on the IK_r or the IK_s component of the current. A protocol used to find out which current is involved in the effects of a particular drug was developed by Noble and Tsien (Noble and Tsien, 1969) and is known as the 'envelope of tail test'. The ratio of tail current relative to that measured during the test depolarization would be constant if *I*K was a simple current (Figure 7). Using this protocol, we found that the ratio tail/total amplitude is not constant under control conditions, being larger at short versus long test pulses. It is well-known that the larger ratio matches the IK_r component of the current, and the smaller one corresponds to IK_s or sustained constituent. In the presence of chlorthalidone, these components were affected in the same proportion: 51% for IK_r and 49% for IK_s, indicating that most probably the drug has no preference for either of the components of the *I*K current.

To clarify which one of the channels is involved in the effect of chlorthalidone, we studied the different proteins (hERG, minK and KvLQT1) that constitute the native delay rectifying potassium current. If the effect of chlorthalidone is on one or all of the components, we will see it in the oocytes transfected with them. Figure 8a shows that the original current records obtained in one oocyte transfected with the three proteins (1:1:1), that form the *I*K current, baseline, perfusion of 100 μM chlorthalidone and after washout. It is evident that the diuretic blocks this current in a similar manner as that in native cells, with less potency in the total amplitude (21%) than in the tail amplitude (91%); these effects are more evident in Figures 8b and c, where the

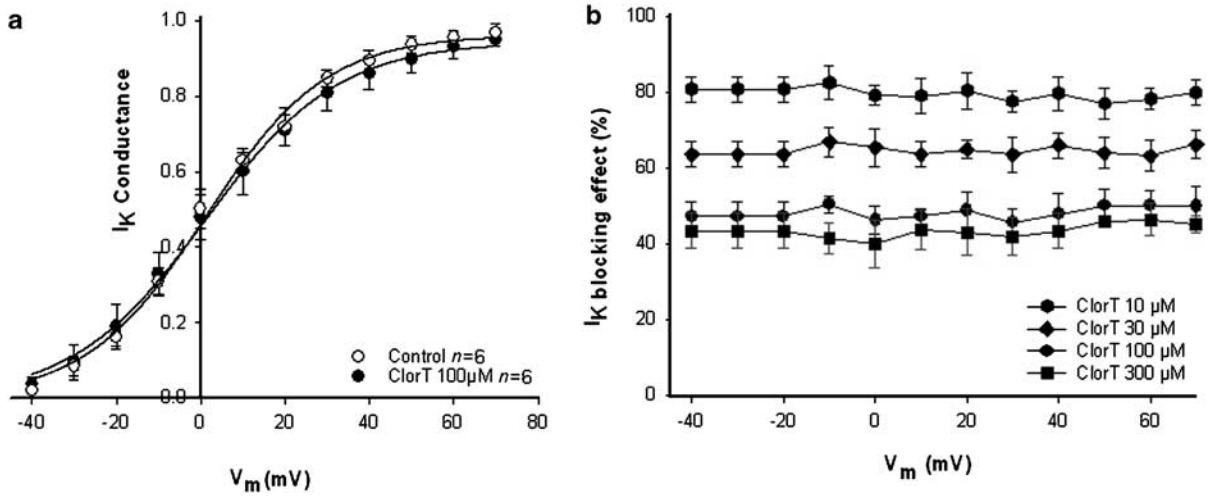

Figure 6 (**a**) Activation curves calculated from the peak tail amplitudes under control conditions and in the presence of 100 μM chlorthalidone. The continuous lines represent the best fit of data to a Boltzmann function. Symbols represent the mean ± s.e.mean of six experiments. (**b**) The plot shows the lack of a voltage-dependent effect by several chlorthalidone concentrations. The mean data represent the average blocking effect on the delayed rectifying potassium current (*IK*) measured at the end of the pulses.

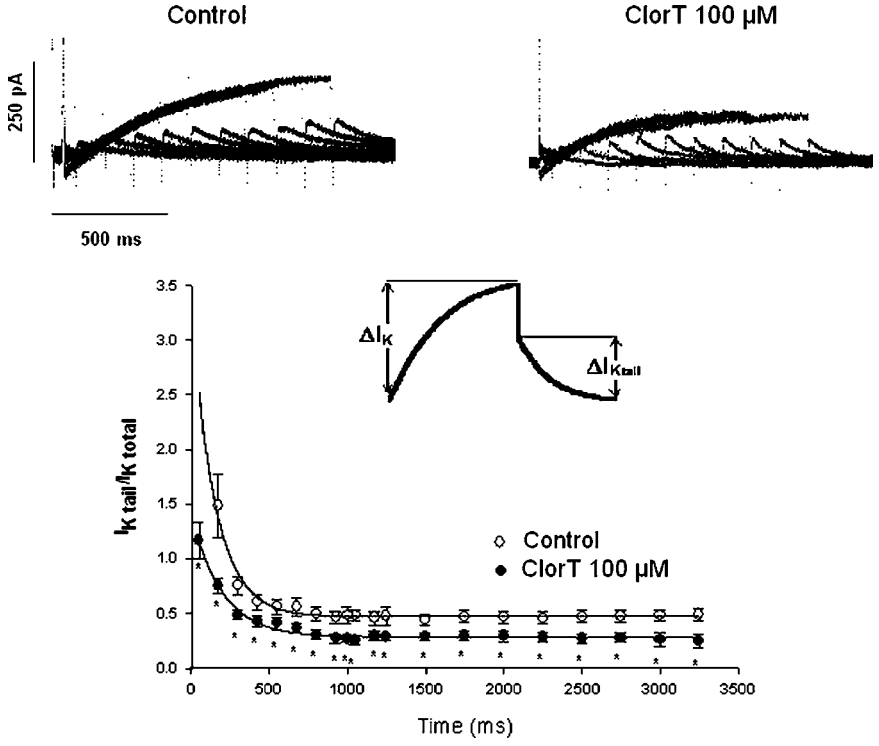

Figure 7 Original current recordings of the tail-test envelope. The test was conducted under two different conditions: control and after the perfusion of 100 μM chlorthalidone. The graph shows the ratio of tail and total currents (IK_{tail}/IK_{total}). In both cases, the resulting ratio is not constant, indicating that the drug affects both currents equally. *$P>0.05$.

current–voltage relationship for four oocytes are summarized. In five oocytes, we also transfected the components one at a time, to determine which one was affected by the drug. MinK channel was not block by chlorthalidone at the concentration examined (100 μM); however, the KvLQT1 channel, the molecular correlate for IK_s current, was partially inhibited by the diuretic, in a similar manner to the oocyte transfected with all three proteins (Figure 8); the total amplitude was reduced by 14%, while the tail amplitude showed more sensitivity to the drug, it was inhibited by 62% (Figure 9). The hERG channel is still under investigation.

Discussion

Our results demonstrate for the first time that, in guinea-pig ventricular myocytes, chlorthalidone lengthens the action potential without affecting its total amplitude or the resting

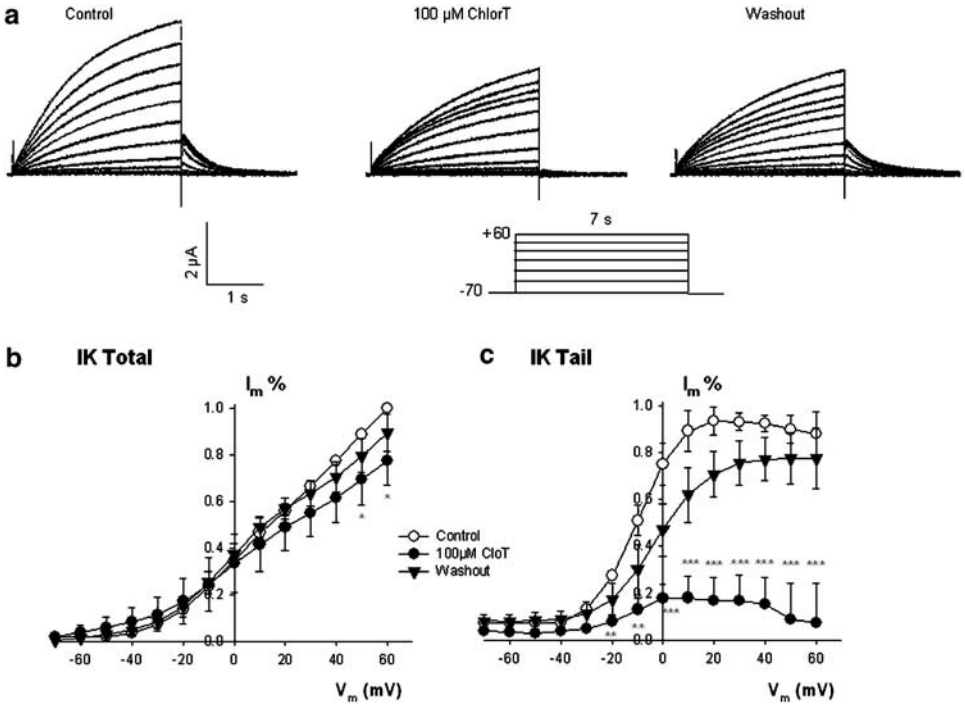

Figure 8 Expression of transfected molecular correlates (human ether a go-go channel (hERG), minimum potassium channel (minK) and potassium long Q-T channel (KvLQT1)) of potassium current clones in *Xenopus oocytes*. (**a**) Original traces showing a set of outward potassium currents elicited by depolarizing pulses from −70 to +60 mV and subsequent deactivating tails at −70 mV, under control conditions, after perfusion of 100 μM chlorthalidone followed by washout of the drug. (**b**) Outward current–voltage relationship for *I*K measured as the difference between the final and initial current during 7-s depolarizing pulses ($n=4$). (**c**) The mean tail current–voltage relationship for three oocytes. Note the strong reduction in the tail current amplitude after the perfusion of the drug. The inset shows the protocol used. *$P>0.05$, **$P>0.01$ and ***$P>0.001$.

membrane potential. To our knowledge, there have been no previous studies published on the direct effects of chlorthalidone on the cardiac action potential.

The effect of chlorthalidone is due to a blockage of the outward I*K* currents, with no significant effect on the I_{to} current or the IK_1 current. It was shown that chlorthalidone, at concentrations of 3–300 μM, exclusively inhibited the *I*K current. In guinea-pig cardiac ventricular myocytes, the *I*K current is produced by the sum of two overlapping components: IK_s and IK_r (Noble and Tsien, 1969; Jurkiewicz and Sanguinetti, 1993). The resulting blockage of any one of those components leads to a prolongation of the action potential. The antiarrhythmic effect of the drugs that act on these currents is a consequence of the lengthening of the cell's refractory period, produced by an increase in the duration of the action potential (VeldKamp *et al*., 1993). On the other hand, this pharmacologically prolonged period of repolarization may cause an acquired long QT syndrome (LQTS) (Spector *et al*., 1996). Long QT syndrome is associated with polymorphous ventricular tachycardia (*Torsade de pointes*), which in turn can lead to ventricular fibrillation and sudden death (Gomez *et al*., 2005).

Chlorthalidone, a benzothiazide, is a diuretic that exerts its action by blocking the Na^+–Cl^- cotransport in the distal convoluted tubules (Ellison *et al*., 1987). Results from previous studies do not accord with our findings as they indicate that chlorthalidone has no electrophysiological effect at all on cardiac potassium currents (Ellison *et al*., 1987; Turgeon *et al*., 1994, 1995). Under our conditions, *in vitro* experiments and no plasma for perfusion of the tissues, we observed a blocking effect of this diuretic, mainly on the I*K* current. This discrepancy in the results may be related to the temperature at which the experiments were conducted. In previous studies, it was demonstrated that in HEK 293 cells transfected with an hERG channel (IK_r in native cells), a change in temperature from 24 to 35 °C produced an eightfold change in activation, inactivation, recovery from inactivation and deactivation time (Hancox *et al*., 1998; Zhou *et al*., 1998). In our case, all the experiments were carried out at a temperature of 36 ± 1 °C, while previous experiments were performed at a temperature of 30 °C or less. The most unexpected result was that, under our experimental conditions, the drug had a very clear dose-dependent effect that could be observed with a concentration of just 5 μM (see Figure 3). However, other researchers (Turgeon *et al*., 1995; Fiset *et al*., 1997) have failed to see any effects despite using concentrations as high as 1 mM.

At concentrations of 100 μM or above, chlorthalidone produced a blocking effect on the total *I*K current after a 7-min perfusion, with a slow recovery after washout (Figure 4b), indicating a slow unbinding within the channels involved in the I*K* current. The IC_{50} for our study was 20.3 μM (Figure 4a), which is equivalent to the average plasma concentration found in humans (5–40 μM; Carter *et al*., 2004) after the administration of a therapeutic dose of chlorthalidone.

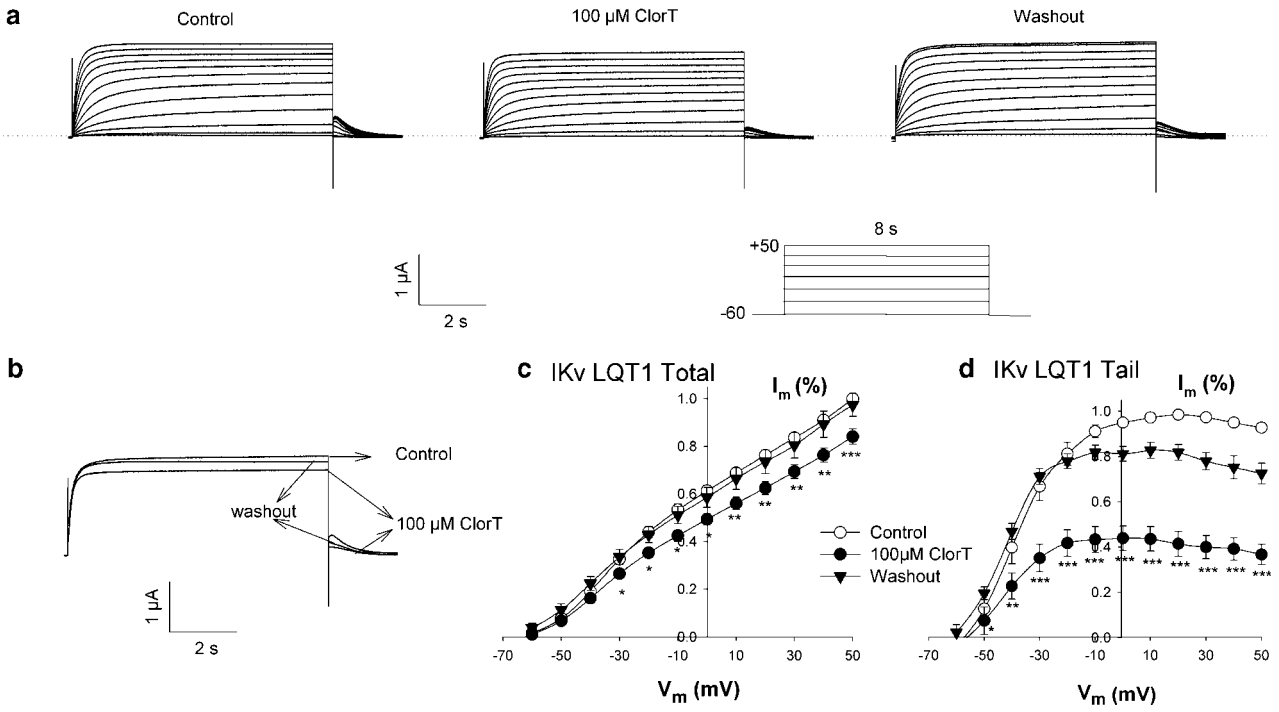

Figure 9 Family of original traces obtained after transfection of the potassium long Q-T channel (KvLQT1) channel. (**a**) Current records elicited by depolarizing pulses from −60 to +50 mV in control conditions, 100 µM chlorthalidone and after washout. (**b**) The current generated at +50 mV pulse superimposed to illustrate the effect of the diuretic is shown. (**c**) The main current–voltage relationship for a total amplitude of the current measured in the same way as that in Figure 8 ($n=5$). (**d**) Current–voltage relationship for the tail current elicited after repolarization to −60 mV. Note the saturation of the current after −30 mV (this has been observed previously for KvLQT1 (Sanguinetti *et al.*, 1996) ($n=5$). *$P>0.05$, **$P>0.01$ and ***$P>0.001$.

The chlorthalidone-sensitive current shows no change in activation kinetics or installation time, indicating the absence of a preferential blocking effect over either of the *I*K components (Figure 5b). However, the chlorthalidone-sensitive current saturated at potentials of +30 mV or higher, indicating a voltage-independent effect (Figure 5c). The measured effect of chlorthalidone after a series of pulses up to +70 mV shows that the current did not change significantly as the voltage was increased (Figure 6b). This effect is not shared by other thiazide diuretics like indapamide (Pickkers *et al.*, 1998; Pickkers *et al.*, 1998) or hydrochlorothiazide (Galán *et al.*, 2001). These results imply that chlorthalidone may bind with several proteins of the channels, and it may explain the same average blocking effect on both components of the current.

In the absence of the drug, the envelope of the tail test closely resembles those previously observed for the *I*K current (Jurkiewicz and Sanguinetti, 1993). Sometimes, it is possible to discriminate the component of the current affected by a drug's actions, as with the effects of E-4031 on the rapid component of *I*K (VeldKamp *et al.*, 1993) and chromanol 293 on the slow IK_s component (Bachmann *et al.*, 2001). In the present study on myocytes, chlorthalidone's blocking effect remained the same after applying the tail test protocol, indicating that it has no selectivity for either component (Figure 7).

At least two types of currents are believed to contribute to repolarization of the cardiac action potential (Jurkiewicz and Sanguinetti, 1993): a rapidly activating, strong rectifying current, IK_r (hERG) and slower current, IK_s (KvLQT1 and minK). When the cDNA encoding these currents were transfected in *Xenopus oocytes*, a current that closely resembles the I*K* current is produced (Figure 8). The addition of the drug produced an effect similar to the one we observed in native cells, indicating that chlorthalidone may bind to these channels. The *I*K current has been shown to be blocked by several drugs, such as quinidine (Roden *et al.*, 1988), amiodarone (Kamiya *et al.*, 2001) and benzenesulphonamides (Sanguinetti *et al.*, 1991), and this effect seems to be an open-channel block. If chlorthalidone acts in a similar manner, its blocking effect should occur when the channel is open (on depolarization), or at least it should increase when the potentials are in a depolarizing direction (open to inactivated state). With chlorthalidone, the current was blocked mainly in positive potentials (> +40 mV), while the tail amplitude (the deactivation of the channel from an open state to the inactivated state) showed a stronger blocking effect (79%) (Figure 8).

When KvLQT1 cDNA (thought to encode, at least in part, IK_s) was expressed in *Xenopus oocytes*, the current produced had the same characteristics as that previously observed for KvLQT1 in oocytes (Pusch *et al.*, 1998). The KvLQT1 outward current was blocked by chlorthalidone in the same manner as the transfected *I*K current (Figure 9). Finally, the minK current was unaffected by the drug (data not shown).

In summary, the results of the present study show that the lengthening of the action potential on myocytes induced by chlorthalidone is partially due to its inhibitory effect on *I*K,

and that this blocking effect may be related to the KvLQT1 component of the current. Also, the inhibition of the current induced by chlorthalidone was voltage-independent. These results support the idea that putative *IK* blockers like chlorthalidone may have important effects on the cardiac repolarization process, particularly in lengthening the cardiac action potential, and they may be useful drugs for preventing the development of arrhythmias. However, it is important that the direct actions of thiazide diuretics on the heart are investigated further, as despite the results presented here, there is substantial evidence indicating that they may increase the risk for fatal arrhythmias due to their effects on potassium concentrations and consequent electrolyte disturbance (Cooper *et al*., 1999).

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Conflicts of interest

The authors state no conflict of interest.

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