Modulation of transient outward current by extracellular protons and Cd^{2+} in rat and human ventricular myocytes

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(Received 9 January 1998; accepted after revision 9 June 1998)

1. The effects of extracellular acidosis and Cd^{2+} on the transient outward current (I_{to}) have been investigated in rat and human ventricular myocytes, using the whole-cell patch-clamp technique.

2. In rat myocytes, exposure to acidic extracellular solution (pH 6·0) shifted both steady-state activation and inactivation curves to more positive potentials, by $20{\cdot}5 \pm 2{\cdot}7$ mV (mean ± S.E.M.; $n = 4$) and $19{\cdot}8 \pm 1{\cdot}2$ mV, respectively. Cd^{2+} also shifted the activation and inactivation curves in a positive direction in a concentration-dependent manner.

3. In human myocytes, the steady-state activation and inactivation curves were located at more positive potentials. The effect of Cd^{2+} was similar, but acidosis had less effect than in rat myocytes (e.g. pH 6·0 shifted activation by only $7{\cdot}2 \pm 2{\cdot}2$ mV and inactivation by $13{\cdot}7 \pm 0{\cdot}5$ mV; $n = 4$).

4. In both species, the effect of acidosis decreased with increasing concentrations of Cd^{2+} and vice versa, suggesting competition between H^+ and Cd^{2+} for a common binding site.

5. The data indicate that acidosis and divalent cations influence I_{to} via a similar mechanism and act competitively in both rat and human myocytes, but that human cells are less sensitive to the effects of acidosis.

Transient outward current (I_{to}) has been identified in cardiac tissues of many species (Kenyon & Gibbons, 1979*a*,*b*; Josephson *et al.* 1984; Clark *et al.* 1988; Dukes & Morad, 1991; Näbauer *et al.* 1993). In some preparations, only one voltage-dependent I_{to} component was found (Dukes & Morad, 1991), whereas in others two components of I_{to}, one Ca_i^{2+}-dependent and one Ca_i^{2+}-independent, were identified (Coraboeuf & Carmeliet, 1982). The Ca_i^{2+}-independent component is carried by K^+ ions and is sensitive to 4-amino-pyridine (Kenyon & Gibbons, 1979*a*,*b*; Coraboeuf & Carmeliet, 1982). On the other hand, extensive evidence indicates that the charge carrier of the Ca_i^{2+}-dependent component is Cl^- (Zygmunt & Gibbons, 1991; Sipido *et al.* 1993). I_{to} plays an important role in the early repolarization phase of the cardiac action potential and its variable regional density underlies the heterogeneity of the action potential within the ventricular wall (Antzelevitch *et al.* 1991).

Extracellular acidosis is known to influence many membrane currents (see Krafte & Kass, 1988; Zhang & Siegelbaum, 1991). Protons and divalent cations induce a shift of voltage-dependent channel gating which is usually explained by a screening of membrane surface charge which changes the potential sensed by the voltage sensor of the channels (see Hille, 1984). However, this theory cannot easily explain the different sensitivity of various channels to divalent ions (Mayer & Sugiyama, 1988; Talukder & Harrison, 1995) nor the wide range of potency of various divalents (Mayer & Sugiyama, 1988; Agus *et al.* 1991; Talukder & Harrison, 1995). Hence the existence of divalent-specific binding sites adjacent to the voltage sensor of the channel has been proposed.

In this report, we have examined and compared the effects of acidosis and the divalent cation Cd^{2+} on I_{to} in rat and human ventricular myocytes, as well as their interaction. Cd^{2+} was chosen as a representative of divalent cations because of its known potency to modulate I_{to} (Agus *et al.* 1991) and because it is often employed as an efficient blocker of L-type Ca^{2+} current ($I_{Ca,L}$) when studying I_{to}. The results show that both acidosis and Cd^{2+} cause a positive shift in activation and inactivation potentials and act in a competitive way; the sensitivity of I_{to} to acidosis is less pronounced in human than in rat ventricular myocytes.

METHODS

Rat cells

The technique for cell isolation was as described previously (Mubagwa *et al.* 1997). In brief, rats were anaesthetized with sodium pentobarbitone (Nembutal; 30 mg kg^{-1}, I.P.) 10 min after having received heparin (500 U, I.P.). Afterwards, the hearts were excised and perfused on a Langendorff apparatus at 37 °C. Cells were dissociated by a protocol which consisted of a constant-flow

perfusion (8–10 ml min^{-1}) for: (1) 5 min with Ca^{2+}-free Tyrode solution; (2) 3 min with Ca^{2+}-free Tyrode solution, containing collagenase (Type A, Boehringer Mannheim; 0·22 mg ml^{-1}) and protease (Type XIV, Sigma; 0·12 mg ml^{-1}); (3) 7–11 min with Ca^{2+}-free, collagenase-containing Tyrode solution; and (4) 10 min with 0·18 mM Ca^{2+}-containing Tyrode solution. The isolated myocytes were stored in normal Tyrode solution at room temperature (22–25 °C).

Human cells

Patients. Myocytes were obtained from eight hearts, six of which were from patients with terminal heart failure due to dilated ($n = 3$) or ischaemic ($n = 3$) cardiomyopathy undergoing transplantation and two were non-failing donor hearts, one which could not be transplanted for technical reasons and a second which was rejected 2 days after transplantation. The mean age of the hearts was 56 ± 6·3 years (means ± S.E.M.). All patients (5 males, 1 female) received digoxin, diuretics and vasodilators.

The investigation was performed according to the Declaration of Helsinki and the protocol was approved by the ethical committee of the University. Informed consent was obtained before organ explantation.

Cell isolation. Part of the left ventricular wall was excised together with its arterial branch. The segment was then perfused via the artery on a Langendorff apparatus at 37 °C at constant flow (8–10 ml min^{-1}) for: (1) 30 min with a nominally Ca^{2+}-free Tyrode solution; (2) 30–40 min with Ca^{2+}-free Tyrode solution containing collagenase (Type A, Boehringer Mannheim, 1 mg ml^{-1}) and protease (Type XIV, Sigma, 0·1 mg ml^{-1}); and (3) 15 min with 0·09 mM Ca^{2+} solution. After the perfusion the tissue was cut into chunks and cells were disaggregated by mechanical agitation in 0·09 mM Ca^{2+}-containing Tyrode (Ca^{2+}-Tyrode) solution. Subsequently, the Ca^{2+} concentration was gradually increased in three to four steps to the final Ca^{2+} concentration (1·8 mM). The cells were stored at room temperature in 1·8 mM Ca^{2+}-Tyrode solution. The yield was approximately 10%. Only cells with clear cross-striations without granulation were selected for experiments.

Voltage clamp

Voltage clamp was performed in the whole-cell configuration of the patch-clamp method using an Axopatch 200A amplifier (Axon Instruments) and heat-polished borosilicate glass pipettes (horizontal puller; Zeitz Instrumente, Germany) with resistances of 2–6 MΩ when filled with pipette solution. Voltage-clamp signals were low-pass filtered (5 kHz 4-pole Bessel), digitized by an A/D converter (Labmaster DMA, Scientific Solutions, Inc., Solon, OH, USA) at 1 kHz (except for measurement of cell capacitance, where the signal was digitized at 100 kHz) and stored in an IBM-AT personal computer using pCLAMP software (Axon Instruments). Series resistances and membrane capacitances were not compensated and capacitive transients decayed with a time constant of 1·0 ± 0·05 ms ($n = 42$) in rat and 1·2 ± 0·08 ms ($n = 28$) in human myocytes. Cell capacitance was calculated from the time integral of the capacitive transient elicited by a 10 mV depolarizing step from the holding potential of −80 mV and was 150·8 ± 7·2 pF ($n = 42$) for rat myocytes and 350·4 ± 29 pF ($n = 28$) for human cells. The rather high value for human cells is probably related to the fact that the cells were mostly obtained from hypertrophied, dilated hearts. Cell capacitance of human cells from healthy donor hearts averaged 212·1 ± 37·6 pF ($n = 7$). Series resistance was estimated by dividing the time constant of the capacitive transient by the cell capacitance and the values were 6·4 ± 0·3 MΩ ($n = 42$) for rat and 4·0 ± 0·4 MΩ ($n = 28$) for human cells. All experiments were performed at room temperature (22–25 °C).

Solutions and drugs

Normal Tyrode solution contained (mM): NaCl, 135; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 0·9; Na_2HPO_4, 0·33; Hepes, 10; and glucose, 10 and the pH was adjusted to 7·4 with NaOH. In the acidic solution, Mes was employed instead of Hepes and pH was adjusted to 6·0. To block $I_{Ca,L}$, nisoldipine (10 μM) or Cd^{2+} (0·1–2 mM) were added. In some experiments, Ca^{2+}-free, 5 mM $MgCl_2$-containing Tyrode solution was used to block $I_{Ca,L}$. Intracellular solution contained (mM): potassium aspartate, 130; KCl, 25; $MgCl_2$, 1; Na_2ATP, 5; EGTA, 1; Hepes, 5; Na_2GTP, 0·1 (pH 7·2, adjusted with KOH). Nisoldipine was from Bayer AG and was prepared as a 20 mM stock solution in dimethyl sulphoxide. Solutions containing nisoldipine were protected from light. Other drugs or chemicals were from Sigma or Merck.

I_{to} measurement

Under our experimental conditions, only the Ca_i^{2+}-independent component of I_{to} was studied since EGTA (1 mM) was included in the pipette solution and $I_{Ca,L}$ was blocked. In most experiments, the holding potential was set at −80 mV from which depolarizing test pulses were made to various levels every 10 s. Test pulses were always preceded by a brief (20 ms) prepulse to −40 mV to inactivate the I_{Na}. The amplitude of I_{to} was measured as the difference between peak current and current remaining at the end of a 900 ms test pulse. Such a method could lead to underestimation of the I_{to} magnitude because the current at 900 ms was still declining. Experiments with longer test pulses revealed that the I_{to} amplitude obtained as the difference between peak current and the current at the end of a 9 s pulse was larger by 15·4 ± 5·7% ($n = 4$). Also, the amplitude of 4-aminopyridine-sensitive current was slightly greater (16·1 ± 3·7%; $n = 4$) than the current obtained as the difference between peak value and value at the end of a 900 ms pulse. However, since the kinetic parameters of I_{to} inactivation were not influenced by either protons or divalent ions (see Results), the subtraction method appears to be reliable enough for the determination of I_{to} amplitude.

Steady-state activation curves were constructed in the following two ways. In most experiments, I_{to} currents at various test potentials were divided by the driving force (taking −70 mV as the reversal potential) and each peak conductance was subsequently normalized to the maximal conductance. The reversal potential (−70 mV) was determined experimentally using a tail current protocol and is in good accordance with the data of others (Apkon & Nerbonne, 1991; Näbauer *et al.* 1993). In some experiments ($n = 4$), I_{to} was activated by a brief step (20 ms) to different potentials and the relative amplitude of the tail current upon return to −20 mV was taken as a measure of I_{to} activation by the first step. The two protocols produced similar results. The inactivation curve was obtained using a two-step voltage-clamp protocol, with a 1 s conditioning step to different potentials followed by a 500 ms test pulse to +60 mV. Inactivation by a given potential was then determined as the ratio of the test current amplitude to the maximal test current. The test current amplitude was obtained as the difference between peak current and the current at the end of the 500 ms pulse (amplitude measured as the difference between peak current and current at 9 s was larger by 26·7 ± 4·3%; $n = 4$). The voltage dependence of both activation and inactivation was fitted by a Boltzmann equation, providing $V_{½}$ (the potential at which half-activation or -inactivation occurs) and slope factor.

Data presentation and statistics

Data are presented as means ± standard error (S.E.M.). Curve fitting with a least-squares method was performed using the software Origin (Microcal Software, Northampton, MA, USA).

When appropriate, Student's paired or non-paired t tests were carried out using Origin.

RESULTS

Rat myocytes

Effect of acidosis

In rat ventricular myocytes, acidification (pH 6·0) of the external solution had a paradoxical effect on I_{to}. Acidosis caused either an increase or decrease of I_{to} or else had no effect at all, depending on both the holding and test potentials. When the holding potential was set to −80 mV, acidosis suppressed the current at most test potentials (Fig. 1*A*) except very positive ones (⩾+60 mV), where acidosis did not elicit any effect (not shown). In contrast, when holding at less negative potentials (−30 mV), the current increased (Fig. 1*B*).

Steady-state activation and inactivation. Such a paradoxical effect could be explained if the voltage dependence of both activation and inactivation was shifted. Steady-state activation and inactivation curves were constructed as described in Methods and are shown in Fig. 1*C*. Acidosis induced a significant rightward shift of both steady-state activation and inactivation curves of I_{to}. Under conditions where $I_{Ca,L}$ was blocked by nisoldipine (10 μM), the $V_{½}$ of the steady-state activation curve was

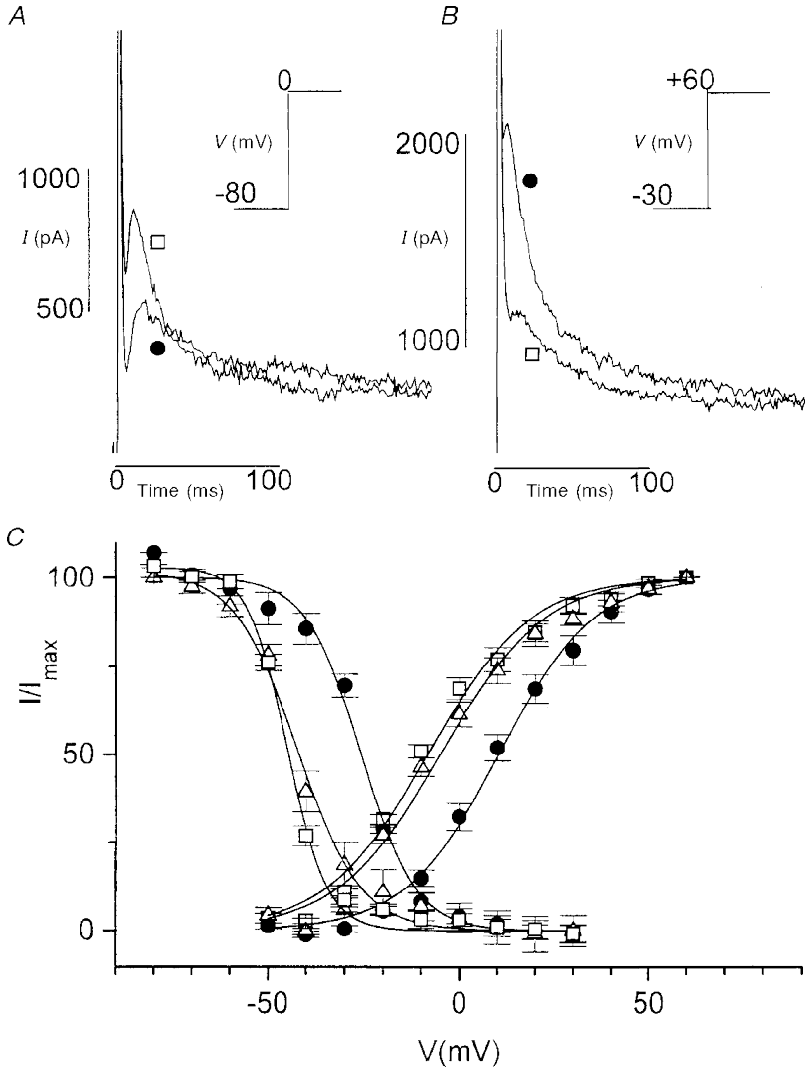

Figure 1. Effect of acidosis (pH 6·0) on I_{to} in rat myocytes

$I_{Ca,L}$ was blocked by nisoldipine (10 μM). *A*, decrease of I_{to} by acidosis. Current activated by a step from a holding potential of −80 to 0 mV. Traces in control conditions (□) and in pH 6·0 (●) are superimposed. Inset, voltage-clamp protocol (20 ms prepulse to −40 mV to inactivate I_{Na} not shown). *B*, increase of I_{to} by acidosis. Current activated by a step to +60 mV after a 1 s prepulse to −30 mV in control (□) and in acidosis (●). Only currents during the test pulse are shown. Inset, voltage-clamp protocol. *C*, effect of acidosis on steady-state activation and inactivation curves. □, control; ●, acidosis; △, second control. The effect of acidosis was reversible.

shifted from $-10{\cdot}6 \pm 0{\cdot}7$ mV ($n = 4$) in control Tyrode solution (pH 7·4) to $+9{\cdot}8 \pm 2{\cdot}3$ mV in acidic solution, and the $V_{½}$ of the inactivation curve was shifted from $-45{\cdot}3 \pm 0{\cdot}9$ mV to $-25{\cdot}5 \pm 0{\cdot}4$ mV. The slope of both curves was not significantly changed ($13 \pm 0{\cdot}9$ in control and $12{\cdot}4 \pm 0{\cdot}6$ in acidosis for the activation curve; $4{\cdot}5 \pm 0{\cdot}3$ in control and $5{\cdot}3 \pm 0{\cdot}1$ in acidosis for the inactivation curve; $n = 4$). The effect of acidosis was completely reversible. In evaluating the $V_{½}$ and the slope of the activation curve it should be realized that the voltage drop due to the series resistance resulted in a rightward shift of the curve and a decrease of the slope; this error was much less for the inactivation curve. The magnitude of the shift is not subject to this error because maximum currents and currents at $V_{½}$ were the same in control and acidic conditions. Since dihydropyridine Ca^{2+} antagonists were reported to inhibit I_{to} and to accelerate inactivation (Gotoh *et al.* 1991), the effect was also examined under conditions where Ca^{2+}-free, 5 mM Mg^{2+}-containing external solution, instead of nisoldipine, was used to block $I_{Ca,L}$. Under these conditions, the shift of activation and inactivation curves was similar: $V_{½}$ of activation was shifted to more positive potentials from $-12{\cdot}8 \pm 4{\cdot}5$ mV by $20{\cdot}9 \pm 2{\cdot}6$ mV and $V_{½}$ of inactivation from $-49{\cdot}8 \pm 2{\cdot}4$ mV by $20{\cdot}6 \pm 0{\cdot}7$ mV ($n = 3$).

Kinetics of activation and inactivation. The time course of I_{to} inactivation was best fitted by two exponentials (Fig. 2*A*, inset). Both the fast time constant ($24{\cdot}7 \pm 2{\cdot}2$ ms at +40 mV with Ca^{2+}-free, 5 mM Mg^{2+}-containing Tyrode solution; $n = 3$) and the slow ($364{\cdot}2 \pm 17{\cdot}7$ ms at +40 mV) time constant were voltage independent (in the range of membrane potentials from 0 to +60 mV) and acidosis did not influence them (Fig. 2*A* and *B*) regardless of the $I_{Ca,L}$ blocker used (nisoldipine, 0 mM Ca^{2+}–5 mM Mg^{2+} or Cd^{2+}). Inactivation was, however, accelerated in the presence of nisoldipine as reported previously by Gotoh *et al.* (1991). The fast time constant was $12{\cdot}3 \pm 1{\cdot}7$ ms and the slow one $85{\cdot}4 \pm 13{\cdot}7$ ms at +40 mV ($n = 4$).

Effect of divalent cations

Since divalent cations are known to shift the voltage dependence of various membrane currents (Hille, 1984) in the same way as acidosis, the effect of Cd^{2+} on I_{to} was also explored. Under conditions where $I_{Ca,L}$ was blocked by 0 mM Ca^{2+}–5 mM Mg^{2+}, addition of Cd^{2+} (0·3 mM) induced an effect similar to that of acidosis. Depending on the holding and test potentials, Cd^{2+} caused either a decrease (Fig. 3*A*) or an increase (Fig. 3*B*) of I_{to}, or had no effect at all (not shown). As with acidosis, the effect was due to a shift of steady-state activation and inactivation curves to more positive potentials (Fig. 3*C*). The $V_{½}$ of the activation curve was shifted from $-13{\cdot}7 \pm 2{\cdot}6$ mV ($n = 3$) to $+8{\cdot}3 \pm 1{\cdot}5$ mV and the $V_{½}$ of the inactivation curve from $-45{\cdot}9 \pm 1{\cdot}2$ mV to $-21{\cdot}2 \pm 3{\cdot}3$ mV. The slope of both curves was not affected ($16{\cdot}6 \pm 1{\cdot}2$ in the absence and $15{\cdot}8 \pm 1{\cdot}1$ in the presence of Cd^{2+} for the activation curve; $4{\cdot}6 \pm 0{\cdot}4$ in the absence and $4{\cdot}4 \pm 0{\cdot}4$ in the presence of Cd^{2+} for the inactivation curve; $n = 3$). The kinetics of I_{to} were changed by Cd^{2+} in the same way as with acidosis. The voltage dependence of the time to peak I_{to} activation was shifted to more positive potentials, consistent with the shift of the steady-state activation

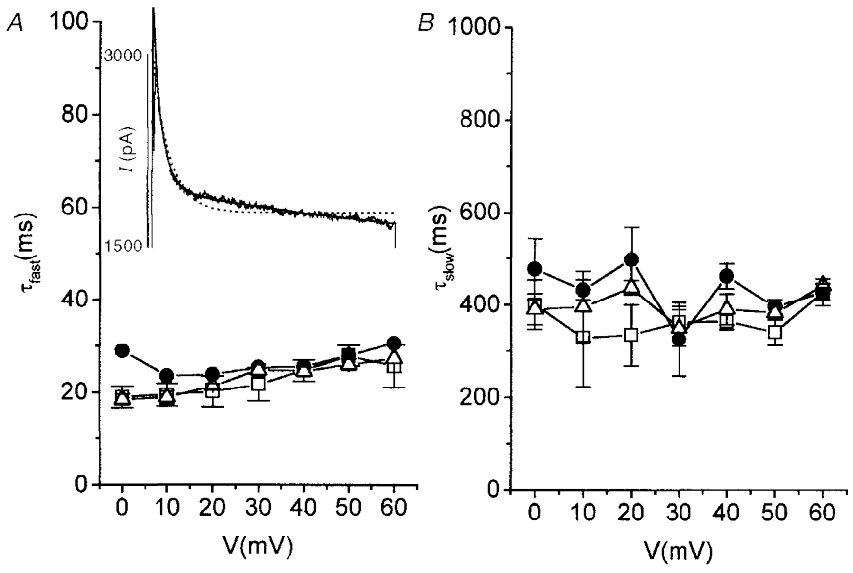

Figure 2. Effect of acidosis on activation and inactivation kinetics in rat myocytes

$I_{Ca,L}$ was blocked by 0 mM Ca_o^{2+}–5 mM Mg_o^{2+}. □, control; ●, acidosis; △, second control. *A*, voltage dependence of the fast time constant of inactivation (τ_{fast}) under control and acidic conditions. Inset, the I_{to} inactivation time course was not satisfactorily fitted by a single exponential (dotted line), but by a double exponential (continuous line, superimposed on the tracing). Current was activated by a 900 ms step to +40 mV from a holding potential of −80 mV. *B* shows the voltage dependence of the slow time constant of inactivation (τ_{slow}) under control and acidic conditions.

curve. The voltage-independent inactivation time constants were not influenced by the presence of Cd^{2+} (data not shown).

Interaction between the effects of pH and of Cd^{2+}

Since the effects of acidosis and divalent Cd^{2+} on I_{to} appeared similar, we next examined their interaction. Figure 4 shows the effect of acidosis in the presence of Cd^{2+} (0·3 mM). Acidosis could either decrease, increase (Fig. 4*A* and *B*) or not change I_{to} (not shown) depending on the holding and test potentials, but the underlying shift of steady-state activation and inactivation curves was less marked in the presence of Cd^{2+}, i.e. the $V_{½}$ of the activation curve was shifted from $+12·2 \pm 3·2$ to $+18·9 \pm 3·2$ mV ($n = 8$) and the $V_{½}$ of the inactivation curve from $-24·9 \pm 1·5$ to $-15·2 \pm 1·3$ mV (Fig. 4*C*). With increasing concentrations of Cd^{2+} (up to 5 mM) the positions of the activation and inactivation curves in control conditions (pH 7·4) were shifted to more positive potentials, and simultaneously the effect of acidosis was decreasing and disappearing at a Cd^{2+} concentration of 5 mM (Fig. 5). On the other hand, the effect of Cd^{2+} was less pronounced in acidic conditions, suggesting a competition for common binding site(s).

Human myocytes

Comparison of outward currents in rat and human myocytes

Outward currents in human myocytes (Fig. 6*A*) were a bit different from those in rat cells (Fig. 6*B*). The sustained current at the end of a 900 ms test pulse was smaller than in the rat ($26·7 \pm 4·7$ % of peak value in human; $n = 16$; and

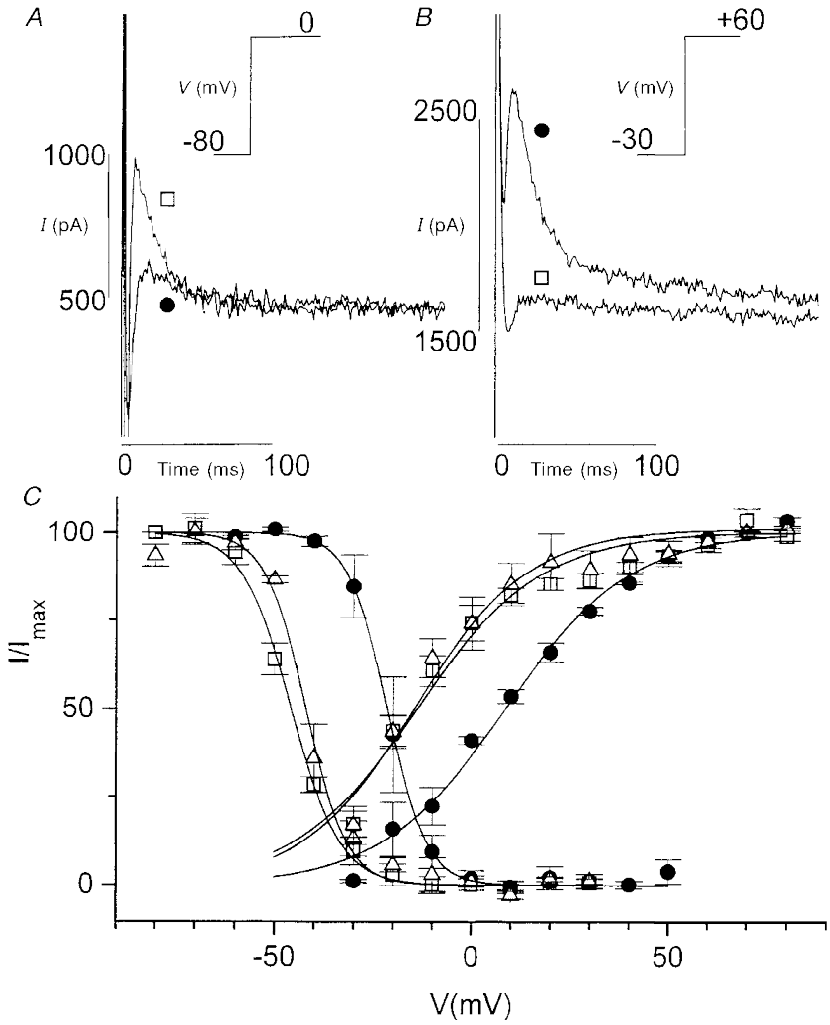

Figure 3. Effect of Cd^{2+} on I_{to} in rat myocytes

$I_{Ca,L}$ was blocked by 0 mM Ca_o^{2+}–5 mM Mg_o^{2+}. *A*, decrease of I_{to} by Cd^{2+} (0·3 mM). Current activated by a step from a holding potential of −80 to 0 mV. Traces in the absence (□) and in the presence of Cd^{2+} (●) are superimposed. Inset, voltage-clamp protocol (20 ms prepulse to −40 mV to inactivate I_{Na} not shown). *B*, increase of I_{to} by Cd^{2+}. Current activated by a step to +60 mV after a 1 s prepulse to −30 mV in the absence (□) and presence of Cd^{2+} (●). Only currents during the test pulse are shown. Inset, voltage-clamp protocol. *C*, effect of Cd^{2+} on steady-state activation and inactivation curves. □, control; ●, Cd^{2+}; △, second control. The effect of Cd^{2+} was reversible.

46·7 ± 2·5 % in rat at a V_m of +60 mV; $n = 29$; $P < 0·001$). The inactivation kinetics were also different since the time course of inactivation was reasonably fitted by a single exponential (time constant of 79·3 ± 11·4 ms; $I_{Ca,L}$ blocked by Cd^{2+}; $n = 6$). Figure 7 shows that both steady-state activation and inactivation curves were at potentials more positive than in the rat. The $V_{½}$ of the activation curve was +0·75 ± 2·9 mV ($n = 4$) and the $V_{½}$ of the inactivation curve was −39·4 ± 0·2 mV in the absence of Cd^{2+}, with nisoldipine as the $I_{Ca,L}$ channel blocker.

Effect of acidosis

The response of I_{to} in human cells to acidosis was qualitatively similar to that in rat myocytes, but less pronounced (Fig. 1*C* and Fig. 7). In the absence of Cd^{2+} (with nisoldipine as the $I_{Ca,L}$ blocker), acidosis shifted the $V_{½}$ of the activation curve only by 7·2 ± 2·2 mV ($n = 4$) and the $V_{½}$ of the inactivation curve by 13·7 ± 0·5 mV. The kinetics of inactivation were both potential (in the range from +20 to +80 mV) and pH insensitive and an acceleration of inactivation was again observed in the presence of nisoldipine (time constant of 31·1 ± 4·4 ms; $n = 4$).

Effect of divalent cations

Application of Cd^{2+} (0·3 mM) induced an effect qualitatively and quantitatively similar to that in rat myocytes. Under conditions where $I_{Ca,L}$ was blocked by nisoldipine, Cd^{2+} shifted both steady-state activation and inactivation curves

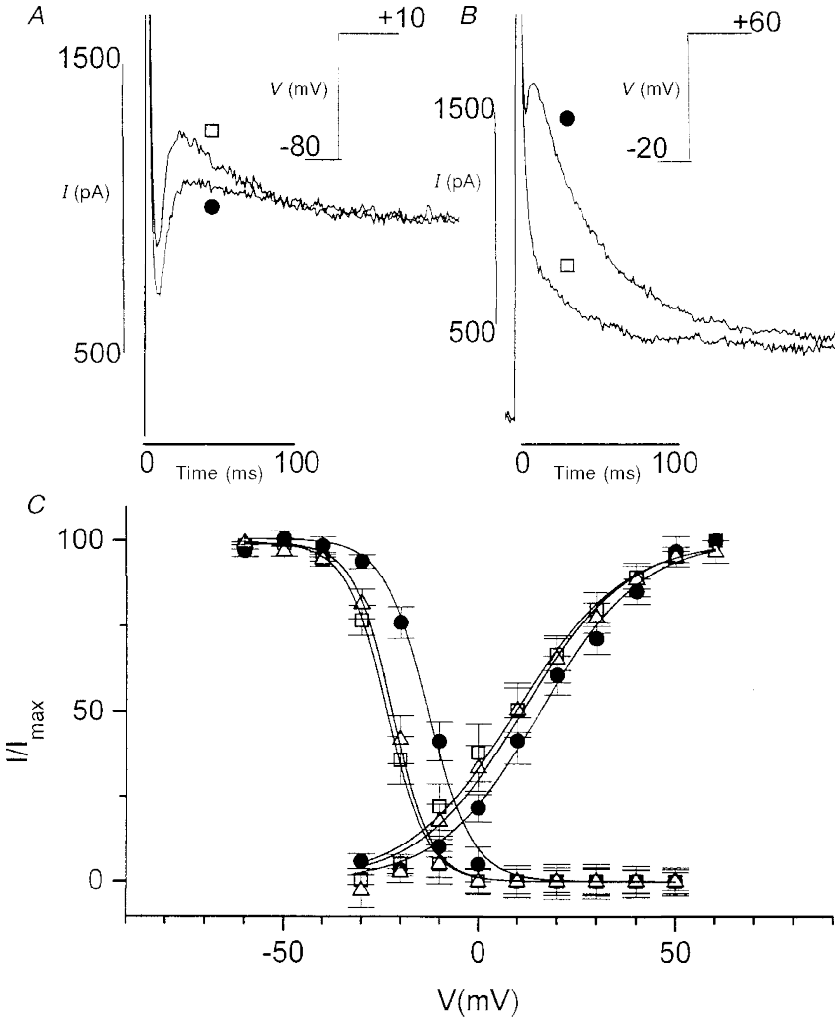

Figure 4. Effect of acidosis on I_{to} in the presence of Cd^{2+} in rat myocytes

$I_{Ca,L}$ was blocked by Cd^{2+} (0·3 mM). *A*, decrease of I_{to} by acidosis. Current activated by a step from a holding potential of −80 to +10 mV. Traces in control conditions (□) and in pH 6·0 (●) are superimposed. Inset, voltage-clamp protocol (20 ms prepulse to −40 mV to inactivate I_{Na} not shown). *B*, increase of I_{to} by acidosis. Current activated by a step from a holding potential of −20 to +60 mV in control (□) and in acidosis (●). Inset, voltage-clamp protocol. *C*, effect of acidosis on steady-state activation and inactivation curves. □, control; ●, acidosis; △, second control. The effect of acidosis was less marked than in the absence of Cd^{2+} (with nisoldipine or 0 mM Ca_o^{2+}–5 mM Mg_o^{2+} to block $I_{Ca,L}$).

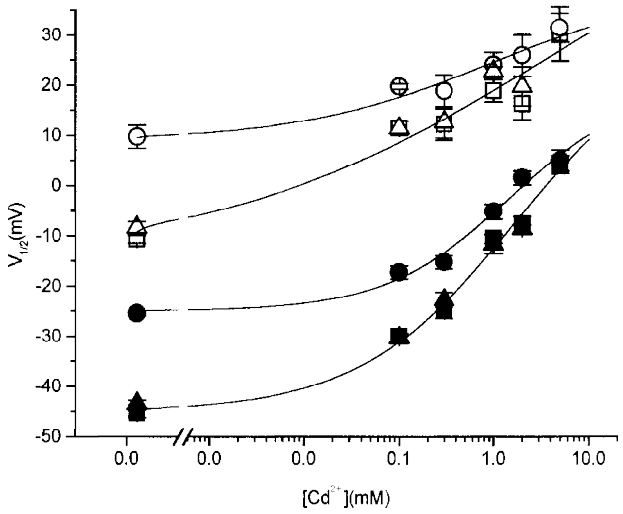

Figure 5. Interaction of acidosis and Cd^{2+} in rat myocytes

Concentration dependence of the effect of Cd^{2+} on the $V_{½}$ of activation (open symbols) or inactivation (filled symbols) in control (squares), acidosis (circles) and second control (triangles). With increasing Cd^{2+} concentration, the $V_{½}$ of both activation and inactivation was shifted to more positive potentials. The effect of acidosis consisted of a further shift of $V_{½}$ to a more positive value but the magnitude of this additional shift decreased with increasing Cd^{2+} concentration. At 5 mM Cd^{2+} acidosis failed to induce a further shift. The values at 0 mM Cd^{2+} were obtained with nisoldipine as the $I_{Ca,L}$ blocker. Different Cd^{2+} concentrations were applied in different groups of cells; n varied between 3 and 8. Data points were fitted by eye (continuous lines).

to more positive potentials by 16·1 ± 1·3 mV ($n = 3$) and 23·2 ± 1·5 mV, respectively (Fig. 8). The kinetics of inactivation were both potential and Cd^{2+} insensitive and decay was again accelerated by nisoldipine (Fig. 8, inset).

Interaction of protons and Cd^{2+}

In the presence of Cd^{2+} (0·3 mM) the activation and inactivation curves were located at more positive potentials ($V_{½}$: +22·2 ± 2·3 mV and −17·0 ± 0·7 mV, respectively; $n = 5$) and acidosis did not induce any further shift of these curves (Fig. 9), a marked difference to rat myocytes, where a significant shift induced by acidosis was still observable at 2 mM Cd^{2+} (see Figs 4 and 5).

DISCUSSION

We have demonstrated that acidosis can either increase or decrease I_{to} or have no effect at all, depending on the holding and test potentials. The depression of I_{to} when activated from potentials negative to −70 mV to potentials less positive than +60 mV is explained by a rightward shift of the activation curve. The absence of an effect of acidosis on I_{to} activated by steps from −80 mV to potentials more positive than +60 mV makes the possibility of a channel block unlikely and further supports the hypothesis that a shift in channel gating underlies the observed I_{to} modulation. On the other hand, acidosis induced a stimulation of I_{to} activated

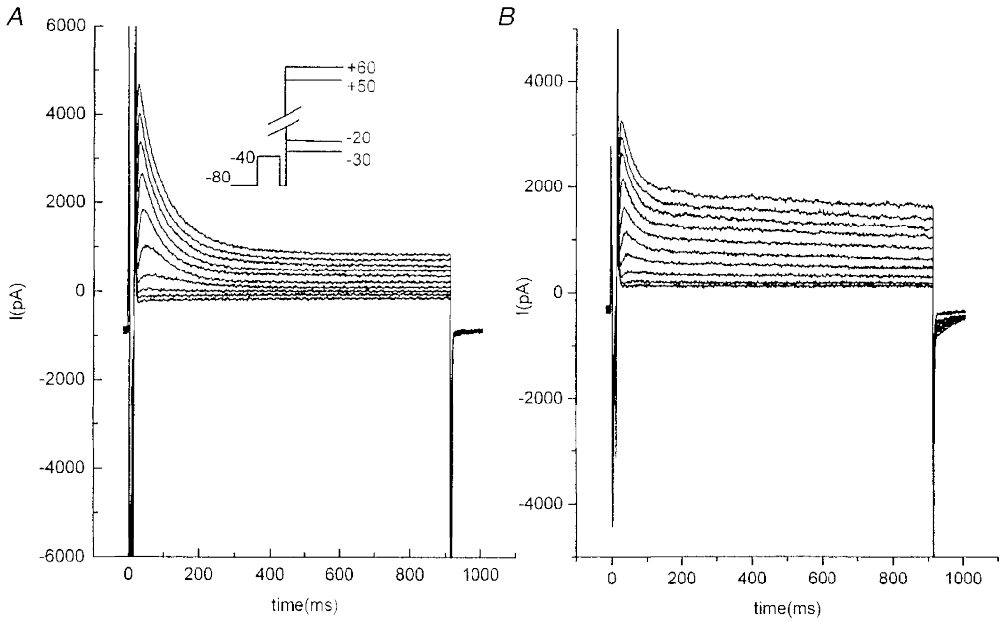

Figure 6. Comparison of outward current in human and rat myocytes

A, depolarization-induced outward current in human ventricular cell. Inset, voltage-clamp protocol: depolarizing pulses were preceded by a 20 ms prepulse to −40 mV to inactivate the I_{Na} current. *B*, depolarization-induced outward current in rat myocytes. Same voltage-clamp protocol as in *A*.

from holding potentials positive to −50 mV (potentials where inactivation is significant) as a result of a positive shift of the steady-state inactivation curve. Therefore, under physiological conditions when the resting membrane potential is negative to −70 mV and the action potential overshoot less positive than +60 mV (as in all ventricular or atrial working cells), acidosis should inhibit I_{to}. However, in preparations such as nodal tissue where the resting membrane potential is under physiological conditions between −50 and −60 mV, or in any cardiac cell when, under pathological conditions, the membrane is depolarized (e.g. during ischaemia by accumulation of external potassium), acidosis will stimulate I_{to} because the negative effect of the rightward activation shift will be overridden by the increased availability of channels due to the shift of the inactivation curve. The stimulation of I_{to} by acidosis when activated from a depolarized potential (−40 mV) was recently reported by Hulme & Orchard (1996). Taken together, acidosis will influence I_{to} differently in different regions of the heart according to the actual resting membrane potential, an action which can alter action potential dispersion and contribute to arrhythmogenesis under ischaemic conditions.

Similar shifts of the voltage dependence of Na^+ and Ca^{2+} channel gating induced either by extracellular protons or by divalent cations have been described in a variety of tissues (Schauf, 1975; Ohmori & Yoshii, 1977; Kostyuk *et al.* 1982; Krafte & Kass, 1988; Zhang & Siegelbaum, 1991; Huang *et al.* 1993; Davidson *et al.* 1995). The phenomenon is usually explained by the surface charge theory, according to which the titration of negative external surface charges leads to changes in surface potential and therefore to changes in the potential sensed by the voltage sensor of the channel. In the original Gouy–Chapman screening model (see Hille, 1984) only the charge of the screening ion was important whereas the improved Gouy–Chapman–Stern model (see Hille, 1984) also takes into account the specific binding of some ions. However, one limitation of this model remains that it assumes a uniform charge (or binding site) distribution, as expected if negatively charged phospholipids were involved. In contrast, studies of Green *et al.* (1987) and of Cukierman *et al.* (1988) in planar lipid bilayers suggest that the negative charge on the channel protein itself is the relevant one, while the membrane phospholipid charge is less important. This was further supported by a report of Mayer & Sugiyama (1988), where manganese induced a significant shift of both steady-state activation and inactivation curves of I_A in cultured rat sensory neurones, but in contrast did not influence voltage dependence of gating of delayed rectifier K^+ current. Similar data were obtained by Talukder & Harrison (1995) in cultured rat hippocampal neurons for various di- and trivalent cations. Whereas our data do not allow us to distinguish between these possibilities, they indicate that the binding sites are accessible for both protons and divalent cations and that protons and divalent cations compete for them.

The characteristics of I_{to} in human and rat myocytes were similar but not identical. One major difference was the position of steady-state activation and inactivation curves. In rat, our observations are consistent with those of others, depending on the $I_{Ca,L}$ channel blocker used (Apkon & Nerbonne, 1991; Lefevre *et al.* 1991; Wettwer *et al.* 1993). The activation and inactivation curves in human cells were found at more positive membrane potentials. This observation corresponds well with the report by Wettwer *et al.* (1993). The difference is probably not related to the fact that human cells were isolated mostly from diseased hearts, since similar activation and inactivation parameters of I_{to} were found in myocytes isolated from ischaemic ($n = 3$), dilated ($n = 3$) and non-failing donor ($n = 2$) hearts. Similarly, Wettwer *et al.* (1993) also did not find any

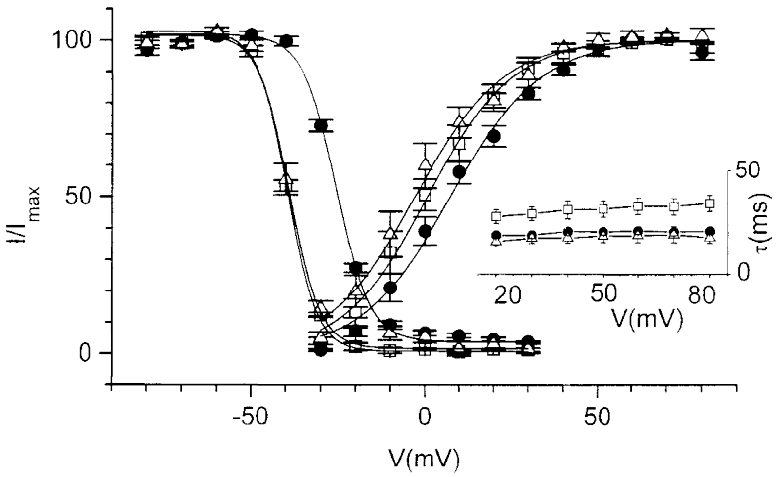

Figure 7. Effect of acidosis on I_{to} in human myocytes

$I_{Ca,L}$ was blocked by nisoldipine (10 μM). Steady-state activation and inactivation curves of I_{to} in control and acidosis are shown. Inset, voltage dependence of the I_{to} inactivation time constant in control and acidosis. □, control; ●, acidosis; △, second control.

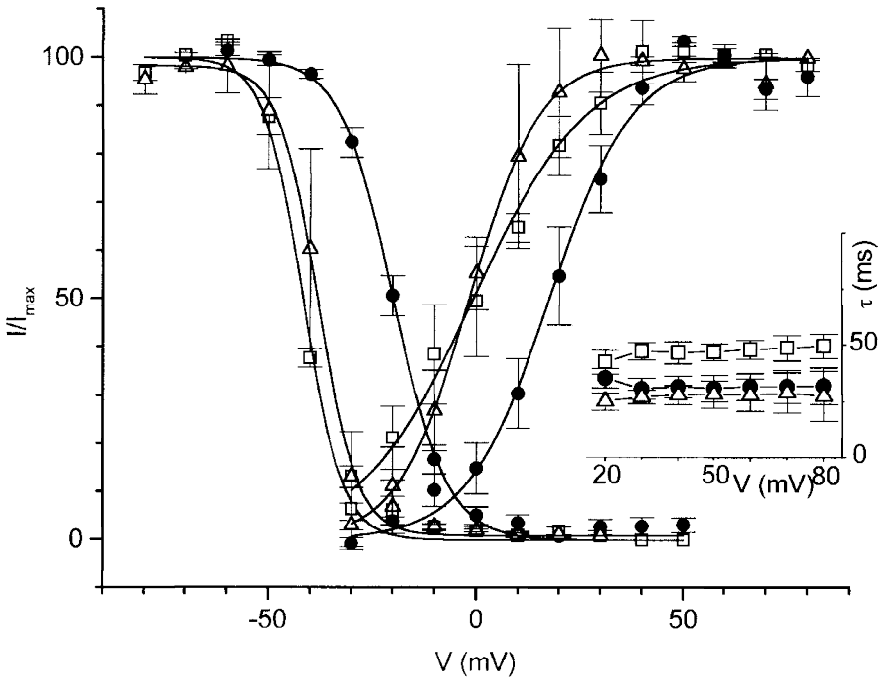

Figure 8. Effect of Cd^{2+} on I_{to} in human myocytes

$I_{Ca,L}$ was blocked by nisoldipine (10 μM). Steady-state activation and inactivation curves of I_{to} in the absence and presence of Cd^{2+}. Inset, voltage dependence of the I_{to} inactivation time constant in the absence and presence of Cd^{2+}. □, control; ●, 0·3 mM Cd^{2+}; △, second control.

difference between human myocytes isolated from diseased and non-diseased hearts. Interestingly, human cells were also much less sensitive to protons while human and rat cells seemed to be equally sensitive to Cd^{2+}. One could speculate that human myocytes from diseased hearts are chronically exposed to ischaemia and therefore they become less sensitive to protons. However, in our experiments similar results were obtained with myocytes from ischaemic, dilated and even from two non-failing donor hearts. Therefore it seems more probable that low sensitivity to protons is a basic property of human cardiac cells, both from diseased and non-diseased hearts. Such a different behaviour should have a morphological substrate and, indeed, it has been reported that Kv4.3 and Kv4.2 channel isoforms are essential components of I_{to} in rat myocytes (Dixon *et al.* 1996; Fiset *et al.* 1997), while only the Kv4.3 isoform underlies the bulk of I_{to} in canine and human heart (Dixon *et al.* 1996). However, further investigation is required to elucidate

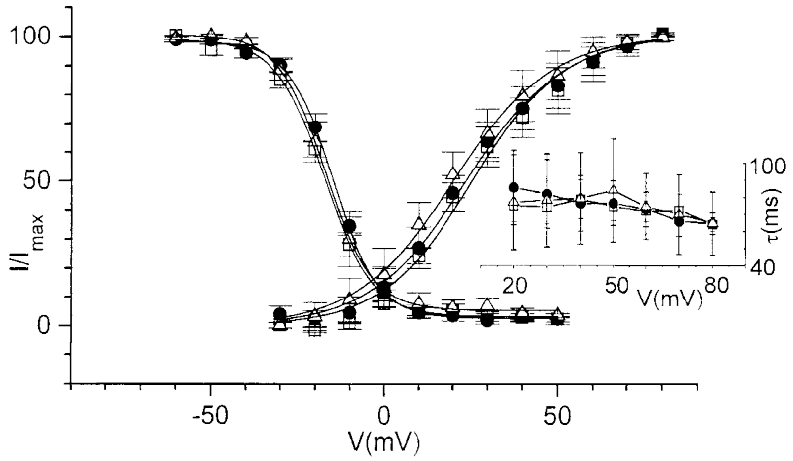

Figure 9. Effect of acidosis on I_{to} in the presence of Cd^{2+} in human myocytes

$I_{Ca,L}$ was blocked by Cd^{2+} (0·3 mM). Steady-state activation and inactivation curves of I_{to} in control and acidosis. Inset, voltage dependence of the I_{to} inactivation time constant in control and acidosis. □, control; ●, acidosis; △, second control.

whether the different sensitivity to external pH is actually due to different channel isoforms underlying I_{to}.

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Acknowledgements

We thank Mr Peter Matejovic for the preparation of rat cells.

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