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Delayed rectifier potassium current in undiseased human ventricular myocytes

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Abstract

Objective: The purpose of the study was to investigate the properties of the delayed rectifier potassium current (I_K) in myocytes isolated from undiseased human left ventricles. **Methods:** The whole-cell configuration of the patch-clamp technique was applied in 28 left ventricular myocytes from 13 hearts at 35°C. **Results:** An E-4031 sensitive tail current identified the rapid component of I_K (I_{Kr}) in the myocytes, but there was no evidence for an E-4031 insensitive slow component of I_K (I_{Ks}). When nifedipine (5 μM) was used to block the inward calcium current (I_{Ca}), I_{Kr} activation was fast (τ=31.0±7.4 ms, at +30 mV, n=5) and deactivation kinetics were biexponential and relatively slow (τ_1 =600.0±53.9 ms and τ_2=6792.2±875.7 ms, at −40 mV, n=7). Application of $CdCl_2$ (250 μM) to block I_{Ca} altered the voltage dependence of the I_{Kr} considerably, slowing its activation (τ=657.1±109.1 ms, at +30 mV, n=5) and accelerating its deactivation (τ=104.0±18.5 ms, at −40 mV, n=8). **Conclusions:** In undiseased human ventricle at 35°C I_{Kr} exists having fast activation and slow deactivation kinetics; however, there was no evidence found for an expressed I_{Ks}. I_{Kr} probably plays an important role in the frequency dependent modulation of repolarization in undiseased human ventricle, and is a target for many Class III antiarrhythmic drugs. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Cell isolation; K-channel; Human myocytes; Ventricular arrhythmias

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1. Introduction

The delayed rectifier potassium current (I_K) is one of the most important transmembrane ionic currents controlling repolarization in mammalian ventricular muscle [1–3]. This current was first described by Noble and Tsien in sheep cardiac Purkinje fibres [4] and has since been identified in various species and cardiac tissue types [4–7]. In most species, I_K consists of two components, I_{Kr} (rapid) and I_{Ks} (slow). These two components differ from each other with respect to their drug sensitivity, rectification and kinetic properties [8–10]. The characteristics of these currents have been extensively studied using the patch-clamp technique in ventricular myocytes obtained from several mammalian species. These studies have revealed important species differences in the existence and properties of I_K [8–13]. Important questions therefore arise as to how these findings may be extrapolated to humans. Because of the known difficulties in obtaining human tissue in general and undiseased human ventricular tissue for research in particular, no studies have so far characterized I_K in healthy human ventricle. The few available data on I_K have been obtained in ventricular myocytes dissociated from diseased human hearts. These data indicate considerable variation that probably reflects differences in the origin of the tissue sample within the ventricle and in cell isolation procedures and experimental conditions [14–18]. Previous studies in ventricular myocytes dissociated from cat hearts with experimentally induced heart failure have shown I_K to be significantly altered [19].

Beuckelmann et al. found that I_K was absent or hardly detectable in human left ventricular myocytes [14], but others verified the existence of I_K in diseased human ventricle [15,16,18]. In a recent study Li et al. described both I_{Kr} and I_{Ks} in right ventricular myocytes obtained

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from explanted human hearts [18]. In this latter study I_K was examined using Cd^{2+} to eliminate the inward Ca current (I_{Ca}) and Ba^{2+} to block the inward rectifier potassium current (I_{k1}). However Cd^{2+} substantially changes the kinetic properties and amplitude of I_K [11,20], so that results obtained in the presence of Cd^{2+} must be interpreted with caution.

The present study provides evidence for the existence of the rapid component of I_K in undiseased human ventricular myocytes. The kinetics for this current are substantially different when either nifedipine or Cd^{2+} is used to block I_{Ca}.

2. Methods

2.1. Patients

Cells were prepared from 13 undiseased donor hearts. The hearts were obtained from general organ donor patients (male=11, female=2; mean age=39.5±13.6 years) undergoing pulmonary and aortic valve transplantation surgery. Before explantation of the hearts the patients did not receive any medication except for dobutamine, furosemide and plasma expanders. The experimental protocol complied with the Declaration of World Medical Association proclaimed in Helsinki and was approved by the Ethical Review Board of the Albert Szent-Györgyi Medical University (No. 51-57/1997 OEj).

2.2. Cell isolation

Ventricular myocytes were isolated from the human hearts by an enzymatic dissociation procedure. After explantation and removal of the valves, hearts were transported in to the laboratory in cold (4°C) cardioplegic solution. A portion of the left ventricular wall was excised together with its arterial branch and was mounted on a modified 60 cm high Langendorff perfusion apparatus, where it was perfused through a branch of the left anterior descending coronary artery with solutions in the following sequence: normal Tyrode's solutions (10 min), Ca^{2+}-free Tyrode's solution (10 min), Ca^{2+}-free Tyrode's solution to which collagenase (type I, 0.66 mg/ml, Sigma Chemical, St. Louis, MO, USA), elastase (type III, 0.045 mg/ml, Sigma Chemical, St. Louis, MO, USA), taurine (50 mM, Sigma Chemical, St. Louis, MO, USA) and bovine serum albumin (fraction V, fatty acid free, 2 mg/ml, Sigma Chemical, St. Louis, MO, USA) had been added (45 min). After this first step of enzymatic digestion the solution was supplemented with protease (type XIV, 0.12 mg/ml, Sigma Chemical, St. Louis, MO, USA) for a further 45–60 min of digestion. Portions of the left ventricular wall that were clearly digested by the enzymes were cut into small pieces and were placed either into Kraft–Brühe (KB) solution, or into Ca^{2+}-free Tyrode's solution supplemented with taurine (50 mM) and $CaCl_2$ (1.25 mM) for 15 min. After this equilibration time the tissue chunks were gently agitated in a small beaker to obtain single cells. The cell suspension contained myocytes of epicardial, midmyocardial and endocardial origin. During the entire isolation procedure, the solutions were oxygenated (100% O_2) and temperature was maintained at 37°C. The cells were allowed to settle to the bottom of the beaker for 10 min, and then half of the supernatant was replaced by new solution. These procedures were repeated three times. The cells in KB solution were stored at 4°C, and the cells stored in Tyrode's solution were maintained on 12–14°C.

2.3. Solutions used for cell isolation

The composition of the solutions was as follows (in mM/l):

1. cardioplegic solution-NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, $NaHCO_3$ 10;
2. normal Tyrode's solution-NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, Glucose 10, $CaCl_2$ 1.0 (pH 7.2 adjusted with NaOH);
3. Ca^{2+}-free solution-NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, Glucose 10 (pH 7.2 adjusted with NaOH);
4. KB solution-KOH 90, L-glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, $MgCl_2$ 0.5, HEPES 10, Glucose 11, EGTA 0.5 (pH 7.3 adjusted with KOH).

2.4. Experimental techniques and solutions

One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (TMS Nikon Co, Tokyo, Japan) and the individual myocytes were allowed to settle to the bottom of the recording chamber for at least 5 min before superfusion was initiated. Only rod shaped cells which showed clear striations were used for study. Altough the yield varied greatly between isolations (from 5 to 70%), the ease of seal formation, the stability of the seals and the quality of the measurements did not correlate with the yield. Cell capacitance (194.0±21.7 pF, n=28) was measured by applying 10 mV hyperpolarizing pulse from a holding potential of −10 mV. The capacity was measured by integration of the capacitive transient divided by the amplitude of the voltage step (10 mV). Action potentials could be recorded from these cells by applying the current-clamp mode of the patch-clamp technique. These were similar to action potentials measured by the conventional microelectrode technique in intact human right ventricular papillary muscles. Hepes buffered Tyrode's solution was used as normal superfusate. This solution contained (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$

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0.53, Glucose 5.5, HEPES 5.0 at pH of 7.4. Superfusion was maintained by gravity flow. E-4031 (obtained as a gift from the Institute for Drug Research, Budapest, Hungary) was prepared freshly daily as a 5 mM aqueous stock solution. A final bath concentration of 5 μM E-4031 was chosen for experiment on the basis of studies showing that this concentration completely blocked I_{Kr} in guinea pig myocytes [8]. Chromanol 293 B (obtained as a gift from the Hoechst AG, Frankfurt, Germany) was also prepared freshly daily in 50% ethanol as a 5 mM stock solution. Micropipettes were fabricated from borosilicate glass capillaries (Clark, Reading, United Kingdom) using a computer controlled horizontal puller (Mecanex, Nyon, Switzerland) and had a resistance of 1.5–2.5 MOhm when filled with a pipette solution containing (in mM) K-aspartate 100, KCl 20, MgATP 5, K_4BAPTA 5, HEPES 10, Glucose 5. The pH of the solution was adjusted to 7.2 with KOH. BAPTA rather than EGTA was used in the pipette solution in order to minimize the possible influence of Na^+/Ca^{2+} exchanger current. The external solution contained 0.25 mM $CdCl_2$ or 5 μM nifedipine in order to completely block the inward Ca^{2+} current (I_{Ca}). The inward sodium current (I_{Na}) was inactivated by applying a holding potential of −40 mV, which largely inactivated the transient outward current (I_{to}) as well. The membrane currents were recorded with an Axopatch-1D amplifier (Axon Instruments, Foster City CA, USA) using the whole-cell configuration of the patch-clamp technique. After establishing high (1–10 GOhm) resistance seals by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1–5 ms. The series resistance was typically 4–8 MOhm before compensation (usually 50–80% depending on the voltage protocols). Those experiments in which the series resistance was high, or substantially increased during the measurements, were discarded from the analyses. The membrane currents were digitized using a 333 kHz analog-to-digital converter (Digidata 1200, Axon Instruments, Foster City CA, USA) under software control (pClamp 6.0, Axon Instruments, Foster City CA, USA). The results were analysed using software programs purchased from Axon (pClamp 6.0, Axon Instruments, Foster City CA, USA) and were low-pass filtered at 1 kHz. When recording action potentials from intact human right ventricular papillary muscle, the conventional microelectrode technique was used as described earlier [21]. The experiments were carried out at 37°C. Statistical analysis was performed by Student's *t*-test for paired data. Numerical data are expressed as mean±SEM.

3. Results

Results were obtained from 28 cells from 13 hearts (average 2.2 cells/heart, range 1–4). We specifically chose to use only cells with high-quality seals and low, stable R_s, opting for a smaller number of excellent cells from each preparation rather than more cells of variable quality. Currents were studied at a holding potential of −40 mV. The cells were depolarized with 1000 ms long test pulses between −20 to +60 mV and than clamped back to −40 mV. The pulse frequency was 0.05 Hz. Since other currents (transient outward, nonspecific cation, chloride and Na^+/Ca^{2+} exchanger currents) could also be activated during depolarizing pulse, the tail current after the end of the test potential was measured to assess I_K (Fig. 1). The amplitude of the tail current was determined as the difference between the peak current during the pulse and the current level at the end of the test pulse. This tail current was completely abolished by 5 μM E-4031 (Fig. 1A) in cells studied in the presence of either nifedipine or $CdCl_2$ (Fig. 1B) indicating that the tail current represented I_{Kr}. Similar results were obtained in 5 out of an additional 5 cells, i.e. we never observed tail current in the presence of E-4031. The amplitudes of the outward current tails after test pulses to different voltages are shown in Fig. 1C. Both curves were fitted by the Boltzmann function, given by $1/[1+\exp(V_m-V_{1/2})/k]$, where V_m is membrane voltage, $V_{1/2}$ is half-activation voltage (−5.74 mV for nifedipine and +13.27 mV for Cd^{2+}) and k is the slope factor (+5.63 mV for nifedipine and +9.23 mV for Cd^{2+}). In the presence of $CdCl_2$ the current-voltage relation was shifted to the right compared to that established in the presence of nifedipine.

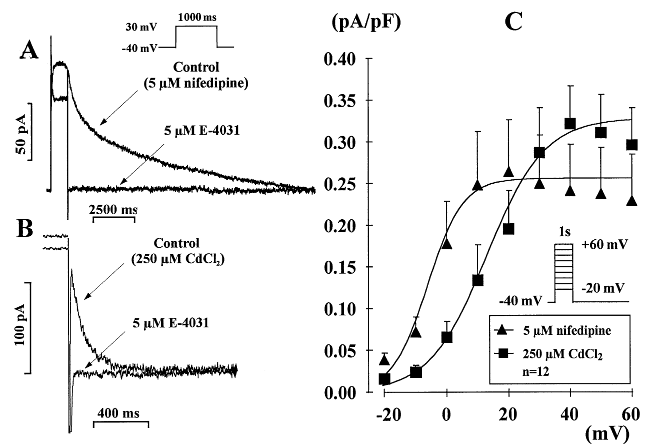

Fig. 1. The effect of E-4031 (5 μM) on the delayed rectifier potassium tail current in undiseased human ventricular myocytes in the presence of either 5 μM nifedipine (panel A) or 250 μM $CdCl_2$ (panel B). Currents were recorded after 1000 ms long depolarizing steps to a +30 mV test potential from a holding potential of −40 mV. Note that the time scales differ between panel A and panel B. Panel C shows the peak current-test voltage relation of the delayed rectifier potassium tail current at −40 mV in undiseased human ventricular myocytes in the presence of either 5 μM nifedipine or 250 μM $CdCl_2$. Both curves were fitted by the Boltzmann function, given by $1/[1+\exp(V_m-V_{1/2})/k]$, where V_m is membrane voltage, $V_{1/2}$ is half-activation voltage (−5.74 mV for nifedipine and +13.27 mV for Cd^{2+}) and k is the slope factor (+5.63 mV for nifedipine and +9.23 mV for Cd^{2+}). Values represent mean±SEM, $n=12$.

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Table 1
Activation and deactivation kinetics for the delayed rectifier potassium tail current in undiseased human ventricular myocytes in the presence of either nifedipine (5 μM) or $CdC1_2$ (250 μM)

Parameters of kinetics	Nifedipine(5 μM)		$CdCI_2$(250 μM)	
	τ_1(ms)	τ_2(ms)	τ_1(ms)	τ_2(ms)
Activation (at +30 mV, n=6)	31.0±7.4	–	657.1±109.1	–
Deactivation (at −40 mV, n=7–8)	599.9±53.9	6792.2±875.6	126.1±15.7	–

τ=time constant in ms.
Values=mean±SEM.

This result indicates that I_K activates at a less positive potential range in the absence of Cd^{2+}. Both current-voltage relations inwardly rectified.

Deactivation kinetics of the tail currents were also distinctly different in the presence of either nifedipine or Cd^{2+} (Fig. 1A and 1B). Thus, with nifedipine the deactivation of I_K-tail was considerably slower than in the presence of Cd^{2+}. Results obtained in seven and eight cells, respectively, for deactivation of the I_K-tails in the presence of nifedipine and Cd^{2+} are summarized in Table 1.

Activation of I_K-tail current was studied with the envelope of tail protocol. Currents were elicited by depolarization from −40 mV to +30 mV with pulses ranging from 10 ms to 5000 ms in duration, and tail currents were recorded after repolarization to −40 mV while pulse frequency was 0.05 Hz. Activation was rapid (31.0±7.4 ms; n=5) in the presence of nifedipine (Fig. 2A) and over an order of magnitude slower (657.1±109.1 ms; n=5) when the cells were instead exposed to Cd^{2+} to block I_{Ca} (Fig. 2B).

The reversal potential of I_{Kr} was measured in the presence of nifedipine by clamping back the cells to voltages between −100 mV to −40 mV after a 1000 ms long depolarizing pulse to +30 mV from a holding potential of −40 mV (Fig. 3). The tail current was determined as E-4031 sensitive current (subtracting the current traces before and after application of 5 μM E-4031) at 4 mM and 8 mM extracellular K^+ concentrations. As Fig. 3 shows, the reversal potential of the current was −83 mV at 4 mM and −65 mV at 8 mM extracellular K^+. These were close to the calculated K^+ equilibrium potential (−90 mV at 4 mM K^+ and −72 mV at 8 mM K^+), strongly suggesting K^+ as the main charge carrier. In two

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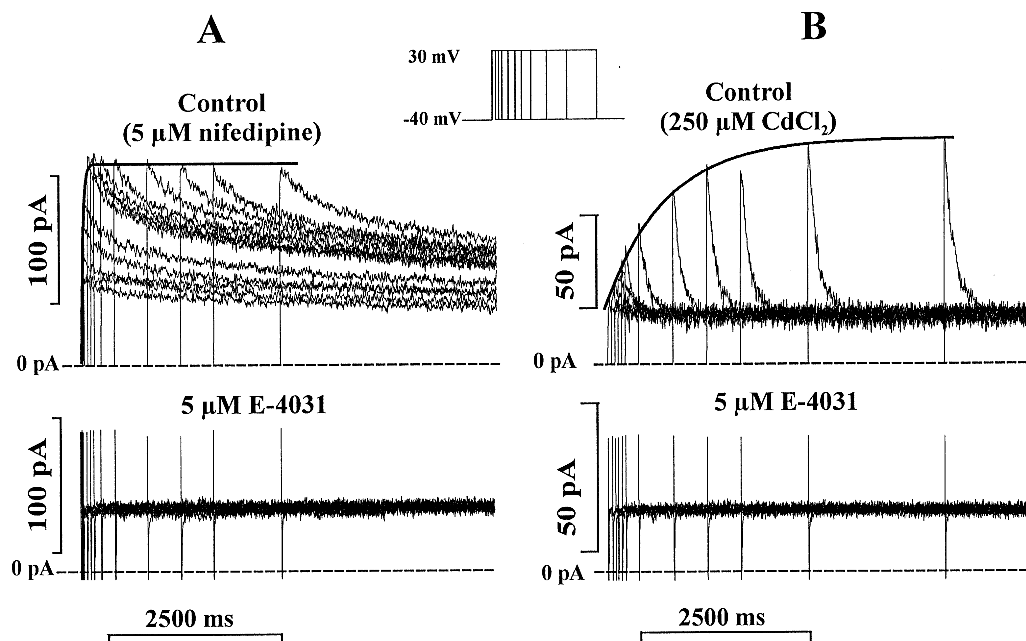

Fig. 2. The effect of E-4031 (5 μM) on the envelope of the delayed rectifier potassium tail currents in undiseased human ventricular myocytes in the presence of either 5 μM nifedipine (panel A) or 250 μM $CdCl_2$ (panel B). Currents were elicited by depolarization from a −40 mV holding potential to a +30 mV test potential with pulses ranging from 10 ms to 5000 ms and tail currents were recorded after repolarization to −40 mV. Pulse frequency was 0.05 Hz.

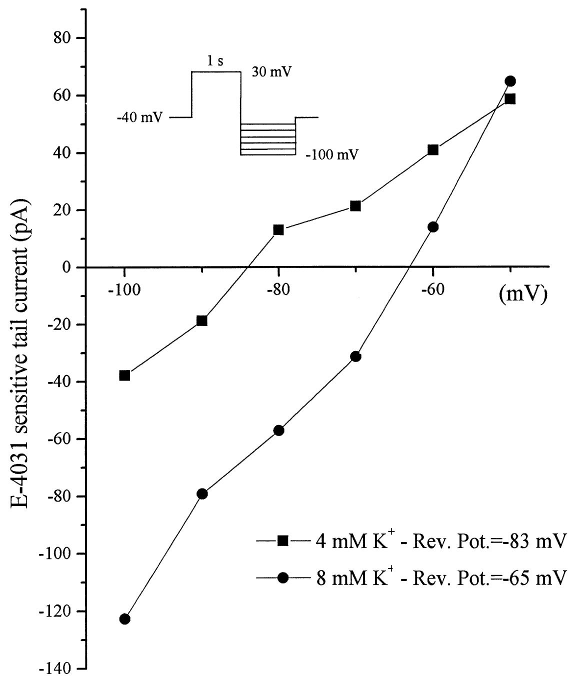

Fig. 3. Reversal potential of the I_{Kr} (defined as E-4031 sensitive tail current). The voltage protocol is shown in the inset. Current values were obtained as the difference in current between measurements made in the absence and presence of E-4031 (5 μM). The extracellular K^+ concentrations were 4 mM (■) and 8 mM (●), respectively.

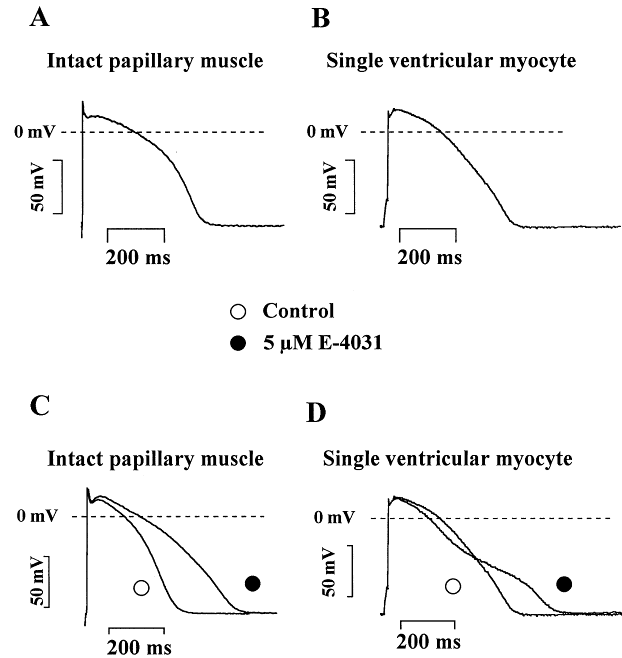

Fig. 4. Action potentials recorded from intact human right ventricular papillary muscle by the conventional microelectrode technique (panel A) and from a left ventricular human myocyte by applying the patch-clamp tehnique in current-clamp mode (panel B). Both experiments were performed in preparations obtained from the same heart. In panel C the effect of E-4031 (5 μM) is shown in the papillary muscle and in panel D the same is demonstrated in single ventricular human myocyte. The temperature was kept constant at 37°C and the stimulation cycle length was 2000 ms.

additional cells at 4 mM K^+ concentration the reversal pontentials were 84.1 mV and 84.8 mV respectively.

As Fig. 4C and D show, 5 μM E-4031 considerably slowed repolarization in human ventricular muscle, measured both in intact human right ventricular papillary muscle by the conventional microelectrode technique and in a left ventricular myocyte by applying the current clamp measurements, suggesting the important role of I_{Kr} in the repolarization of the human ventricle.

In a recent study in myocytes dissociated from explanted right human ventricle, both I_{Kr} and I_{Ks} were described in the presence of Cd^{2+} and Ba^{2+} [18]. In our experiments tail currents were not detected after application of 5 μM E-4031 in 12 cells, suggesting that I_{Ks} was not present in these myocytes. Since activation of I_{Ks} is facilitated by elevating intracellular cAMP, 3 cells were exposed to 5 μM forskolin (Fig. 5) and I_K-tail was measured in the presence of 5 μM E-4031. Forskolin increased steady-state outward currents, probably reflecting changes in cAMP dependent Cl-conductances, but still no I_K-tail was recorded in the presence of forskolin. This finding further suggests that in our experiments the I_K-tail represents I_{Kr}, with no evidence for the existence of an expressed I_{Ks}. When I_K-tail was measured in the presence of $CdCl_2$, the amplitude was increased by superfusion with 500 μM $BaCl_2$ (Fig. 6A). Under these conditions, and after addition of 5 μM E-4031, a small tail current component was observed (Fig. 6B). This residual tail current resembled

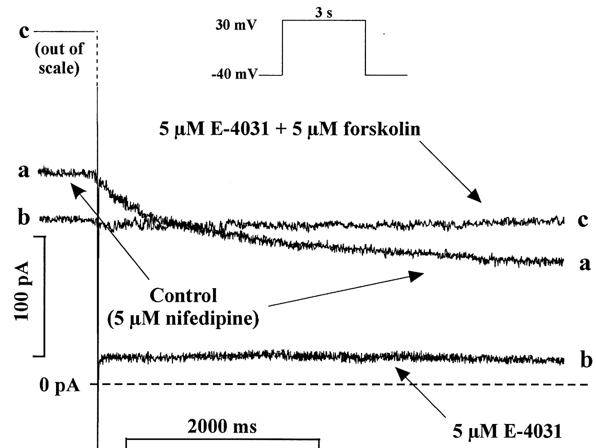

Fig. 5. The lack of the slow component of the delayed rectifier potassium tail current in an undiseased human ventricular myocyte. In control conditions, in the presence of 5 μM nifedipine (a), and after a 3 s depolarizing step to +30 mV from a holding potential of −40 mV, an outward tail current was observed after repolarization to −40 mV. E-4031 (5 μM) completely abolished this current (b). Although addition of 5 μM forskolin to the tissue bath activated a steady-state outward current (c) (probably due to an increase in a cAMP dependent Cl-conductance), no outward tail current was observed.

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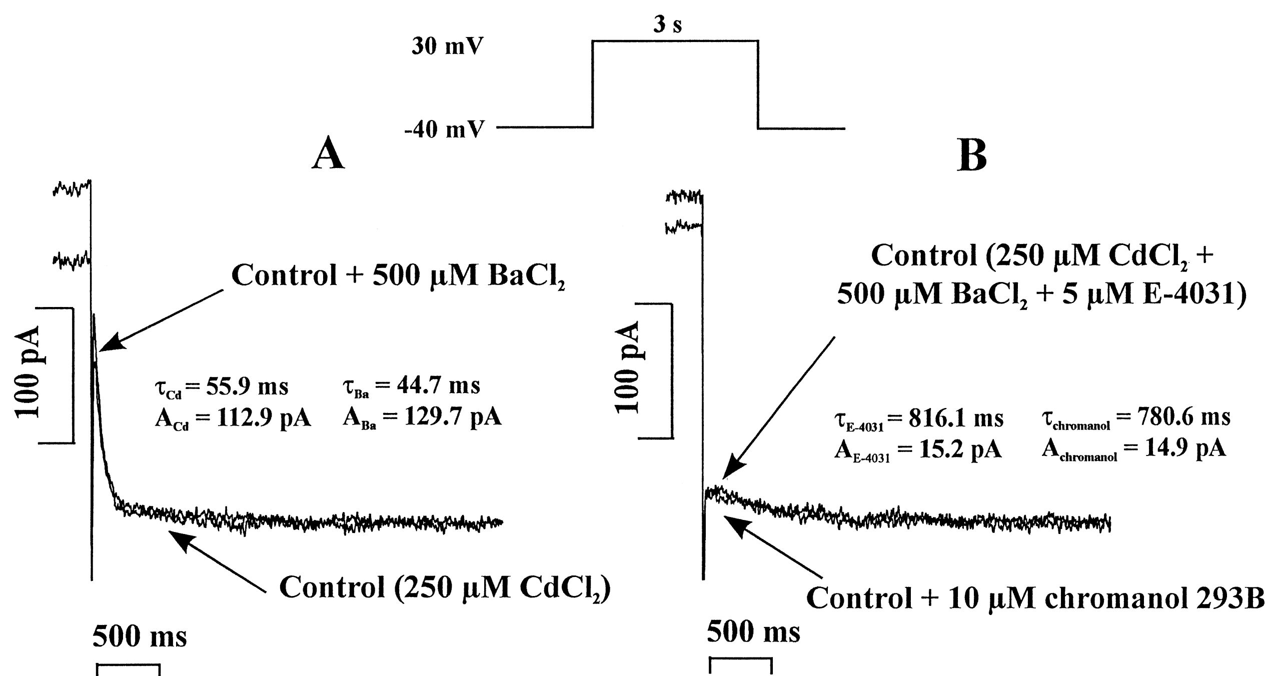

Fig. 6. Influence of $BaCl_2$ on the outward tail current in an undiseased human ventricular myocyte following block of the inward calcium current (I_{Ca}) by 250 μM $CdCl_2$. $BaCl_2$ (500 μM) enhanced the outward tail current recorded following a 3 s depolarization step to +30 mV from a holding potential of −40 mV (panel A). After superfusion of the myocyte with E-4031 (5 μM) in the presence of the $BaCl_2$, a small residual tail current was observed (panel B). This small E-4031 resistant outward tail current was not changed by superfusion of the myocyte by 10 μM chromanol 293B (purportedly a selective I_{Ks} blocker). The corresponding deactivation time constants (τ) and amplitude values (A) of the presented tail currents are given in the inset.

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that described as I_{Ks} by Li et al. [18] although it was smaller. Superfusing the cell with 10 μM chromanol 293 B, a purported selective blocker of I_{Ks} [22], did not change this tail current. Thus, it is questionable if the Ba^{2+}-induced residual tail current could indeed be attributed to I_{Ks}. Similar results were obtained in two additional cells. Since in our hands the amplitude of the Ba^{2+}-induced tail current was small, no further attempt was made to characterize it.

4. Discussion

In this study I_{Kr} was identified and characterized in undiseased human ventricle. This current exhibited fast activation and slow deactivation kinetics. These kinetic properties were shown to be greatly influenced by Cd^{2+}. In addition, there was no evidence found of an expressed I_{Ks} in left ventricular cells obtained from undiseased human hearts.

In our study, identification and characterization of the I_{Kr}-tail current was based on earlier experimental observations suggesting that the E-4031 sensitive tail current represents I_{Kr} [8]. Activation and deactivation kinetics of I_K are reported to vary greatly in mammalian cardiac cells depending on the species and area of the heart from which they were obtained [7–9,11,13]. The kinetic properties of human I_{Kr} were discussed in earlier studies carried out in diseased cardiomyocytes. Recently Li et al. [18] measured the I_{Kr} activation time constant, which proved to be 192 ms, and Beuckelmann et al. [14] reported similar values, but in both studies Cd^{2+} was used to block I_{Ca}. We found that Cd^{2+} substantially altered the kinetic properties of I_{Kr}. In the study by Veldkamp et al. [15] the activation time constant was 101 ms and similar values were obtained by Wang et al. in human atrial myocytes (≈90 ms) [23].

The properties of I_{Kr} in our study best resemble those observed by Gintant in dog ventricular myocytes [9]. In Gintant's study nisoldipine was used to block I_{Ca} and I_{Kr} was found to activate quickly (within 50 ms), while it deactivated slowly ($\tau \approx 2000$ ms). In the dog ventricular myocytes examined by Gintant however, there was also a substantial E-4031 insensitive tail current. This current was attributed to I_{Ks} and was not observed in the present study in human ventricular cells.

Our data provide several new insights into the function of I_{Kr} in undiseased human ventricular myocytes and extend earlier observations made in explanted diseased hearts [15,18] by establishing that I_{Kr} also exists in undiseased human ventricle. Earlier studies carried out in diseased cardiomyocytes either did not show data regarding the kinetics of I_{Kr} or were performed in the presence of Cd^{2+} and Ba^{2+} [14,18]. However, our results indicate that in normal human ventricular myocytes divalent cations substantially alter the characteristics of I_K as noted earlier by others in cats and guinea pigs [11,20].

We did not find evidence for the existence of I_{Ks}, consistent with the observations of Veldkamp et al. [15] and Konarzewska et al. [16]. One recent report, however, described I_{Ks} in right ventricular myocytes obtained from explanted diseased human hearts [18]. This discrepancy may be due to differences in the techniques used to enzymatically disperse the myocytes. Alternatively the difference could be related to the finding of Hirano et al. in guinea pig ventricular muscle who described development of an outward tail current after application of $BaCl_2$ [24]. These authors postulated that the Ba^{2+}-induced block of I_{k1} dissapeared at positive potentials and slowly developed again upon return to more negative holding potentials to produce an apparent tail current which resembled I_K. Our results support this speculation, since we only observed an outward tail current following application of $BaCl_2$ in the presence of E-4031 to block I_{Kr}. This tail current, that resembled I_{Ks}, was also resistant to chromanol 293B, a selective blocker of I_{Ks} in guinea pig ventricular myocytes [22].

4.1. Implications

The present findings have two major implications. First, we demonstrate the importance of the modulating effect of two commonly used divalent cationic channel blockers (Cd^{2+} and Ba^{2+}) on I_K: Cd^{2+} slowed activation of I_{Kr} and accelerated its deactivation while Ba^{2+} induced a small E-4031 insensitive tail current resembling I_{Ks} although it was not blocked by chromanol 293B. Second, our data clearly indicated that I_{Kr} is well expressed in undiseased human ventricle and therefore it probably plays an important role in repolarization. The fast activation and relatively slow deactivation kinetics of I_{Kr} characterized in the absence of Cd^{2+} suggest that in human this current modulates the frequency dependent changes in repolarization. The lack of I_{Ks} and the slow deactivation kinetics of I_{Kr} provide further experimental evidence to support the suggestion by Gintant based on his dog studies [9] that, in contrast to the hypothesis relating to findings in the guinea pig [8,25], I_{Kr} may play a significant role in the rate-dependent action potential abbreviation. The reverse use-dependent effects of I_{Kr} blockers are thus not easily explained by a relatively greater role of I_{Ks} at fast rates (contrary to the suggestion based on data obtained in the guinea pig [25]. Undoubtedly, further investigation is necesary to reveal the exact mechanism of reverse use-dependence.

Acknowledgements

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