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Cardiovascular Research 49 (2001) 790–797

Cardiovascular Research

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The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes

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Received 28 January 2000; accepted 21 November 2000

Abstract

Objective: The purpose of this study was to investigate the properties of the slow component of the delayed rectifier potassium current (I_{Ks}) in myocytes isolated from undiseased human left ventricles. **Methods:** The whole-cell configuration of the patch-clamp technique was applied in 58 left ventricular myocytes from 15 hearts at 37°C. Nisoldipine (1 μM) was used to block inward calcium current (I_{Ca}) and E-4031 (1–5 μM) was applied to inhibit the rapid component of the delayed rectifier potassium current (I_{Kr}). **Results:** In 31 myocytes, an E-4031 insensitive, but L-735,821 and chromanol 293B sensitive, tail current was identified which was attributed to the slow component of I_K (I_{Ks}). Activation of I_{Ks} was slow (τ=903±101 ms at 50 mV, n=14), but deactivation of the current was relatively rapid (τ=122.4±11.7 ms at −40 mV, n=19). The activation of I_{Ks} was voltage independent but its deactivation showed clear voltage dependence. The deactivation was faster at negative voltages (about 100 ms at −50 mV) and slower at depolarized potentials (about 300 ms at 0 mV). In six cells, the reversal potential was −81.6±2.8 mV on an average which is close to the K^+ equilibrium potential suggesting K^+ as the main charge carrier. **Conclusion:** In undiseased human ventricular myocytes, I_{Ks} exhibits slow activation and fast deactivation kinetics. Therefore, in humans I_{Ks} differs from that reported in guinea pig, and it best resembles I_{Ks} described in dog and rabbit ventricular myocytes.

Keywords: Cell culture/isolation; K-channel; Membrane currents; Myocytes; Ventricular arrhythmias

1. Introduction

The delayed rectifier potassium current (I_K) is an important repolarizing current in the ventricle [1,2] that has been identified in various mammalian cardiac preparations [3–6]. In most species, I_K can be separated into rapid (I_{Kr}) and slow (I_{Ks}) components that differ from one other in terms of their sensitivity to drugs, rectification characteristics and kinetic properties [7–9]. Since the properties and relative magnitude of I_{Kr} and I_{Ks} show species and tissue dependent variation the question arises as to how the findings obtained in experimental animals may be extrapolated to humans. In spite of the well known difficulties in obtaining human cardiac tissue for research purposes, there have been several reports of I_K in human ventricular muscle. The I_{Kr} current was first described in man by Veldkamp et al. [10] and by Li et al. [11] and it was further characterized by Iost et al. [12].

The available data regarding I_{Ks} in human ventricular muscle are less certain than the available information on I_{Kr} as was thoroughly discussed in a recent editorial by Veldkamp [13]. In right ventricular myocytes obtained from explanted hearts, Li et al. [11] described an E-4031 insensitive and indapamide-sensitive current, which was attributed to I_{Ks}. These measurements were, however,

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Time for primary review 24 days.

0008-6363/01/$ – see front matter
PII: S0008-6363(00)00306-0

performed in the presence of $CdCl_2$ and $BaCl_2$ making the interpretation of the results difficult. A previous report from our laboratory noted that it was not possible to identify I_{Ks} current in myocytes obtained from undiseased human left ventricle [12]. Although this latter study verified the presence of properties of I_{Kr}, it raised further questions about the existence of I_{Ks} in undiseased human ventricular muscle [12].

However, in recent experiments using different experimental conditions (changing the content of pipette and extracellular solutions) we were able to record an E-4031-insensitive, L-735,821- and chromanol 293B-sensitive current in undiseased human ventricular myocytes. In the present study we describe the properties of this current, which we attribute to I_{Ks}.

2. Methods

2.1. Patients

Cells were prepared from 15 undiseased donor hearts. The hearts were obtained from general organ donor patients (male=7, female=8; mean age=44.3±4.3 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 as described previously [12]. After explantation and removal of the valves, hearts were transported 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: isolation solution supplemented with 1 mM $CaCl_2$ (10 min), isolation solution (10 min), isolation solution to which collagenase (type I, 0.66 mg/ml, Sigma Chemical, St. Louis, MO, USA), elastase (type III, 0.045 mg/ml, Sigma) and bovine serum albumin (fraction V, fatty acid free, 2 mg/ml, Sigma) had been added (15 min). After the first step of enzymatic digestion, the solution was supplemented with protease (type XIV, 0.12 mg/ml, Sigma) for a further 30–45 min. 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 isolation solution supplemented with taurine (50 mM) and $CaCl_2$ (1.25 mM) for 15 min. The tissue chunks were then gently agitated in a small beaker to obtain single cells. 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 isolation solution (1.25 mM $CaCl_2$) were maintained on 12–14°C. The characteristics of I_{Ks} current recorded from cells stored in KB solution did not differ from those stored in isolation solution. At least 1 h was allowed before the start of the experiments.

2.3. Solutions used for cell isolation:

The composition of the solutions was as follows (in mM): (a) cardioplegic solution: NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, $NaHCO_3$ 10; (b) isolation solution (Ca^{2+}-free): NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, Na pyruvate 5, taurine 20, $NaHCO_3$ 4.4, glucose 10 (pH 7.2 adjusted with NaOH); (c) 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. Only rod-shaped cells, which showed clear striations, were used. Although the yield varied greatly between isolations (from 5 to 70%), the ease with which seals were formed, the stability of the seals and the quality of the measurements did not correlate with yield. Cell capacitance (182±15 pF, n=19) was measured by applying 10 mV hyperpolarizing pulse from −10 mV. The holding potential was −90 mV. The capacity was measured by integration of the capacitive transient divided by the amplitude of the voltage step (10 mV). 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, $MgCl_2$ 0.53, glucose 5.5, and 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. Final bath concentrations of 1–5 μM E-4031 were chosen for experiments on the basis of studies showing that these concentrations completely blocked I_{Kr} in guinea pig myocytes [7]. 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. L-735,821 (obtained as a gift from Merck-Sharpe & Dohme, NY, USA) was prepared in 100% DMSO as a 1-mM stock solution and was used at 100 nM in order to completely block I_{Ks} as reported in dog [14]. Micropipettes were fabricated from borosilicate glass capillaries (Clark, Reading, UK) using a microprocessor controlled horizontal puller (Sutter Instruments Co., Novato CA, USA) and had a resistance of 1.5–2.5 MOhm when filled with a solution containing (in mM) KOH 100, KCl 20, K_2HPO_4 10, $MgCl_2$ 5, K_2EGTA 5, HEPES 10, Na_2ATP 5, KADP 0.5, Na pyruvate 5, glutamate 5, creatine 5, glucose 5. The pH of the solution was adjusted to 7.2 with aspartic acid. The external solution contained 1 μM nisoldipine (kind gift form Bayer Hungary Ltd, Budapest) in order to completely block the inward Ca^{2+} current (I_{Ca}). To increase the amplitude of I_{Ks} most of the experiments were carried out in the presence of 1 μM forskolin. 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 Axopatch-1D and Axopatch-200B patch-clamp amplifiers (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 analysis. 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 and 7.0, Axon Instruments). The results were analyzed using software programs purchased from Axon (pClamp 6.0 and 7.0, Axon Instruments) and were low-pass filtered at 1 kHz. The experiments were carried out at 37°C. Numerical data are expressed as mean±S.E.M.

3. Results

Results were obtained from 31 cells from 15 hearts (average 2.1 cells/heart, range 1–4). We specifically chose to use only cells with high-quality seals and low, stable series resistance (R_s), opting for a smaller number of excellent recordings from each preparation rather than more recordings of variable quality. In additional 27 cells we could either not observe I_{Ks} or the I_{Ks} amplitudes were so small that it was difficult to distinguish the current from the noise.

Fig. 1A shows tail currents elicited by test pulses to 50 mV with gradually increasing duration (from 10 to 5000 ms). In this particular experiment the pulse frequency was 0.05 Hz. This was necessary because the deactivation of I_{Kr} in human myocyte is very slow [12]. However, in the rest of the study where I_{Kr} was abolished by E-4031, we applied a pulse frequency of 0.1 Hz which allowed for the E-4031 insensitive tail current to be fully deactivated. The current was activated with a double exponential time course (τ_{fast}=22.4 ms, τ_{slow}=2388 ms) suggesting that it represented both I_{Kr} and I_{Ks} currents. Complete block of I_{Kr} by 5 μM E-4031 markedly reduced the amplitude of the tail currents and changed its activation to a single exponential (τ=1763 ms) with a slow time course (Fig. 1B). Additional superfusion of the cell with 30 μM chromanol 293B, a blocker of I_{Ks}, entirely abolished this residual tail current suggesting that this current indeed represented I_{Ks} (Fig. 1C). All of the other experiments on I_{Ks}, therefore, were performed in the presence of 1 or 5 μM E-4031 to completely block I_{Kr}, thereby facilitating the separation of I_{Ks} from I_{Kr}.

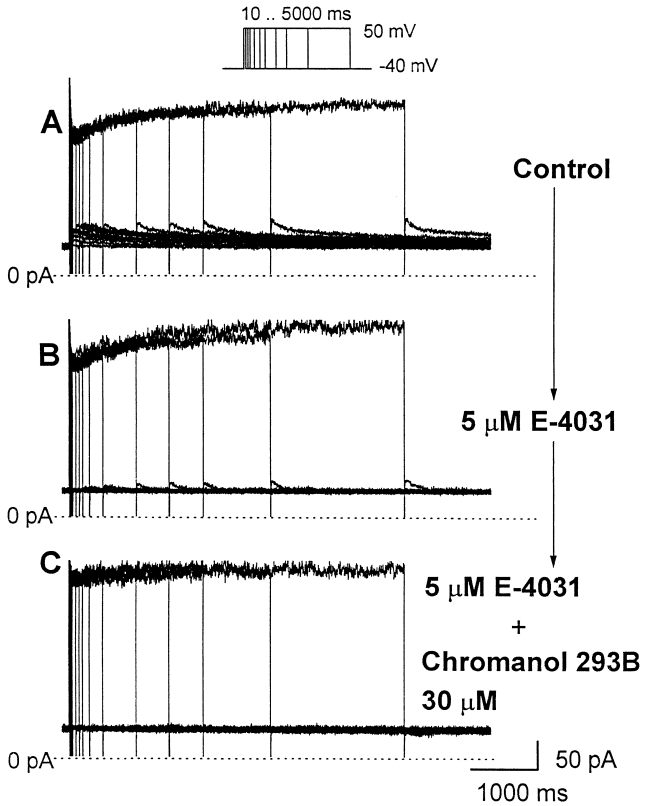

Fig. 1. Two components of the delayed rectifier potassium current in human ventricular myocytes. Representative current traces were recorded under control conditions (panel A), after application of 5 μM E-4031 (panel B) and in the presence of 30 μM chromanol 293B and 5 μM E-4031 (panel C). The current was activated by depolarizing voltage pulses to 50 mV with gradually increasing duration (from 10 to 5000 ms) at pulse frequency of 0.05 Hz. The holding potential was −40 mV. The dotted line shows zero current level.

Since I_{Ks} is relatively small, and other currents (transient outward, non-specific cation, chloride and Na^+/Ca^{2+} exchanger currents) could be also activated during de-

polarizing pulses and during the time course of experiments, the tail current after the end of the test pulse was measured to assess I_{Ks}.

An instantaneous, background-like, outward current was often developed in long lasting (45–90 min) measurements (see Figs. 1 and 2). This may represent a current activated because of the dialysis between the pipette and the intracellular milieu.

Fig. 2A and B show representative current traces in the presence of 1 μM E-4031 under control conditions and after application of 100 nM L-735,821, respectively. The inset in Fig. 2A indicates that the I_{Ks} tail current deactivated rapidly (τ=122.4±11.7 ms, from 50 mV, n=19) and was completely eliminated by L-735,821, another more specific blocker of I_{Ks} (see Fig. 2B). Fig. 2C indicates average data obtained in 12–14 cells showing the I_{Ks} tail current normalized to the cell capacitance in respect to gradually increasing voltages. Currents were studied at a holding potential (HP) of −40 mV. The cells were depolarized with 5000 ms long test pulses between −10 and +50 mV and then clamped back to −40 mV. Tail current amplitude was measured as the difference between the peak and the steady state current level at the end of the pulse after returning back to −40 mV HP.

Fig. 3 shows the activation kinetics of I_{Ks} tail current. To determine the activation kinetics of I_{Ks} we used the envelope of tails test protocol. From holding potential of −40 mV test pulses to 50 mV were applied with increasing duration ranging from 100 to 5000 ms and the amplitude of the tail currents was measured. Fig. 3 contains the result

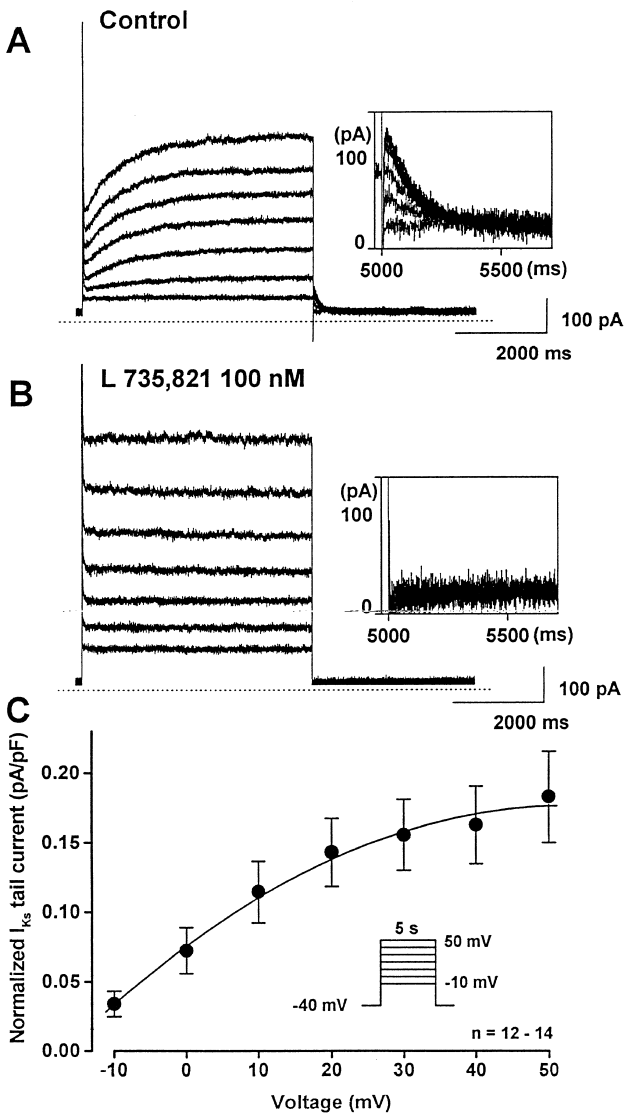

Fig. 2. Representative current traces under control conditions (panel A) and after application of 100 nM L-735,821 (panel B). The current was activated by 5000 ms long depolarizing voltage pulses from holding potential of −40 mV to various test potentials ranging from −10 to 50 mV in 10-mV increments (see inset in panel C). The pulse frequency was 0.1 Hz. The insets in panel A and B indicate the tail currents of the original traces. Panel C indicates the cell size normalized current–voltage relationship for I_{Ks} tail current expressed in pA/pF (n=12–14). In all, 5 μM E-4031 was present throughout the measurements.

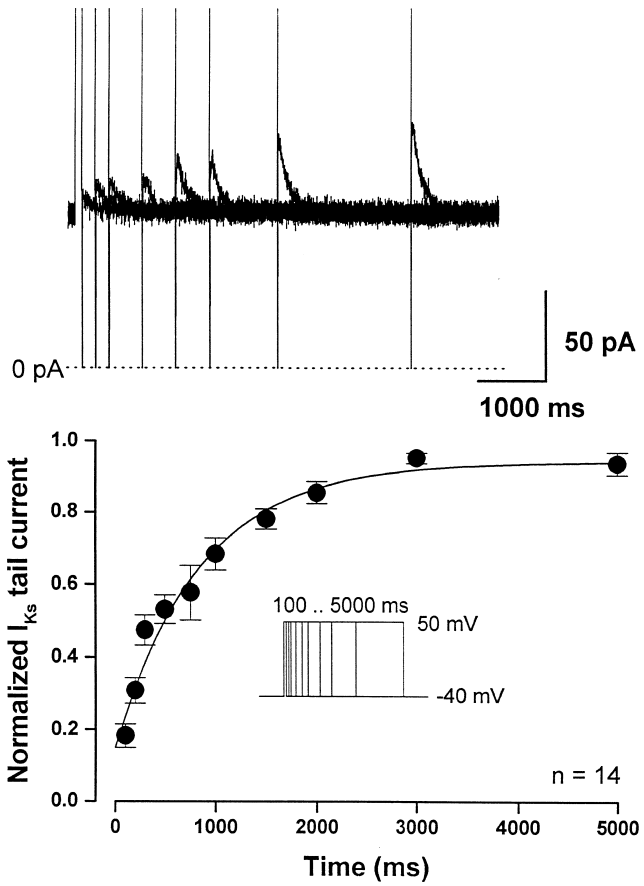

Fig. 3. The activation kinetics of human I_{Ks} tail current. Original current traces (top) and the normalized I_{Ks} tail current amplitude as a function of test pulse duration (bottom) are shown. The curve was fitted by a single exponential function in order to show the calculation of the activation time constant. The activation kinetics of the current was studied by using test pulses with gradually increasing duration at 50 mV (see inset). The amplitude of I_{Ks} tail current was normalized to the maximum amplitude value recorded from the particular cell.

of a representative experiment, indicating that activation of the tail current was rather slow (903±101 ms at 50 mV, $n=14$). In some cells, the voltage dependence of activation of I_{Ks} was also studied. The activation kinetics of the I_{Ks} tail current measured at −40 mV was apparently not voltage dependent in the test voltage range of 10–60 mV (Fig. 4A and B).

The voltage dependence of the deactivation kinetics of I_{Ks} current was determined by the following protocol: I_{Ks} current was activated by 5-s long test pulse to 50 mV from a holding potential of −40 mV. Then the cells were clamped back to different voltages ranging from −50 to 0 mV. Fig. 4C indicates that the deactivation of I_{Ks} tail current was clearly voltage dependent, i.e. being faster at negative voltages (about 100 ms at −50 mV) and slower at depolarized potentials (about 300 ms at 0 mV).

The reversal potential of I_{Ks} was measured by clamping the cells back to voltages between −100 and 0 mV after a 5000-ms long depolarizing pulse to 50 mV from a holding potential of −40 mV. The results of a representative experiment are shown in Fig. 5. The tail current in these measurements was determined as L-735,821 sensitive

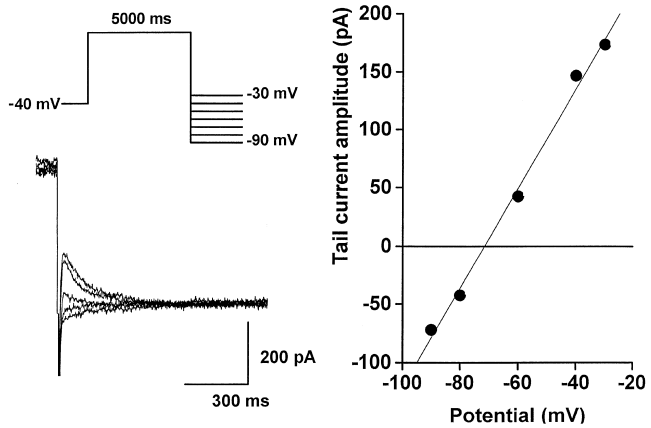

Fig. 5. The reversal potential of human I_{Ks} was measured by clamping the cells back to voltages between −90 and −30 mV after a 5000-ms long depolarizing pulse to 50 mV from a holding potential of −40 mV (top trace left). The tail current in these measurements was determined as L-735,821 sensitive current (subtracting the current traces before and after the application of 100 nM L-735,821) (bottom trace left). Linear regression was applied concerning the data point negative to −40 mV. The reversal potential was calculated as the intersection of the fitted and the zero current lines (right).

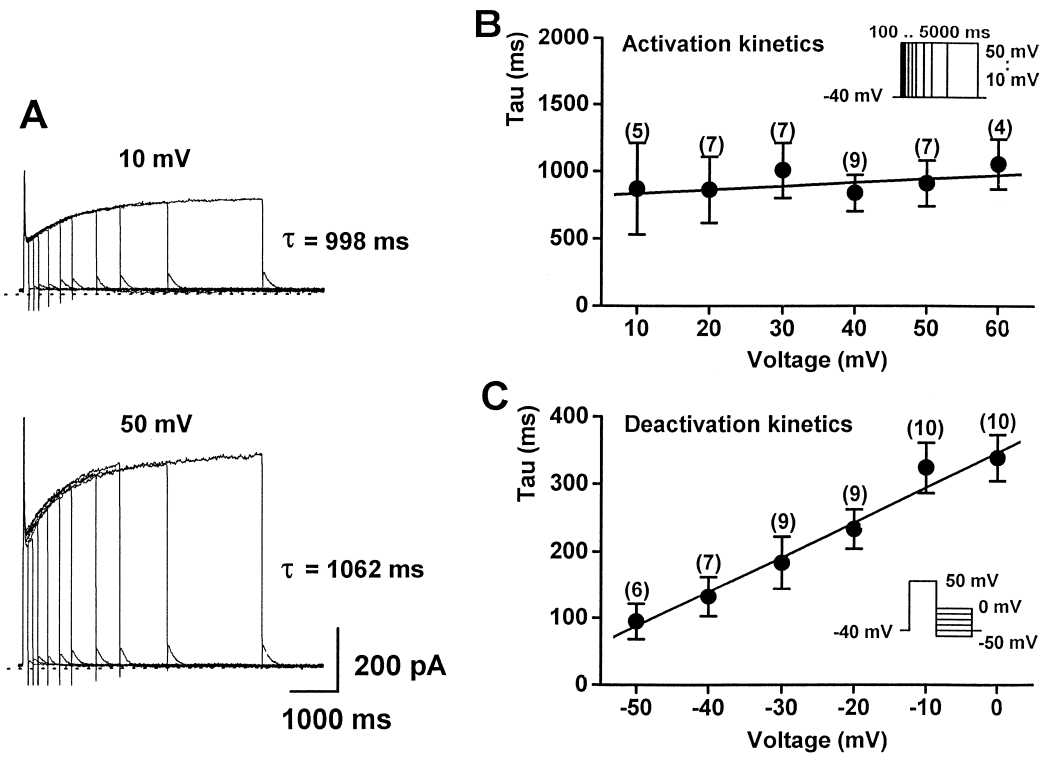

Fig. 4. The voltage dependence of the activation and deactivation kinetics of human I_{Ks}. Original current traces illustrate the activation kinetics of human I_{Ks} at 10 mV (top) and 50 mV (bottom) test potentials (panel A). Activation kinetics as a function of the test potential (panel B) were measured by envelope of tails protocol (see inset). I_{Ks} current was activated by test pulses with duration from 100 to 5000 ms to various test potentials ranging from 10 to 60 mV, then the cells were clamped back to −40 mV. The amplitude of tail current as a function of the duration of the depolarizing test pulse was well fitted by a single exponential function. The voltage dependence of the deactivation kinetics (panel C) was determined by using the voltage protocol indicating the inset in panel C. I_{Ks} current was activated by 5000-ms long test pulse to 50 mV from holding potential of −40 mV. Then the cells were clamped back to different potentials ranging from −50 to 0 mV and the deactivation time course of the tail current was fitted by a single exponential function.

current (subtracting the current traces before and after application of 100 nM L-735,821). In six cells, the reversal potential was -81.6 ± 2.8 mV on an average which is close to the K^+ equilibrium potential suggesting K^+ as the main charge carrier.

Li et al. [11] reported a relatively large (about 200 pA) I_{Ks} tail current in myocytes obtained from the right ventricle in failing human hearts. In this latter study the extracellular solution contained Cd^{2+} and Ba^{2+} to block I_{Ca} and I_{K1}. These divalent ions, however, markedly alter the kinetics of I_K [12,15] and in particular Ba^{2+} blocks I_{K1} in a voltage-dependent manner [16]. Therefore, in some cells we also measured 'I_{Ks}' in the presence of 0.5 mM $BaCl_2$. As Fig. 6 shows, in the presence of the selective blocker of I_{Kr} (1 μM E-4031) and I_{Ks} (100 nM L-735,821), as expected, no tail current was recorded at −40 mV after a step potential to +30 mV for various durations. Cells were then exposed to 0.5 mM $BaCl_2$ that elicited a slowly developing tail current that resembled I_{Ks}, as it was also found in two other cells. However, since both I_{Ks} and I_{Kr} were completely blocked by 100 nM L-735,821 and 1 μM E-4031 this current could not be due to the activation of I_K.

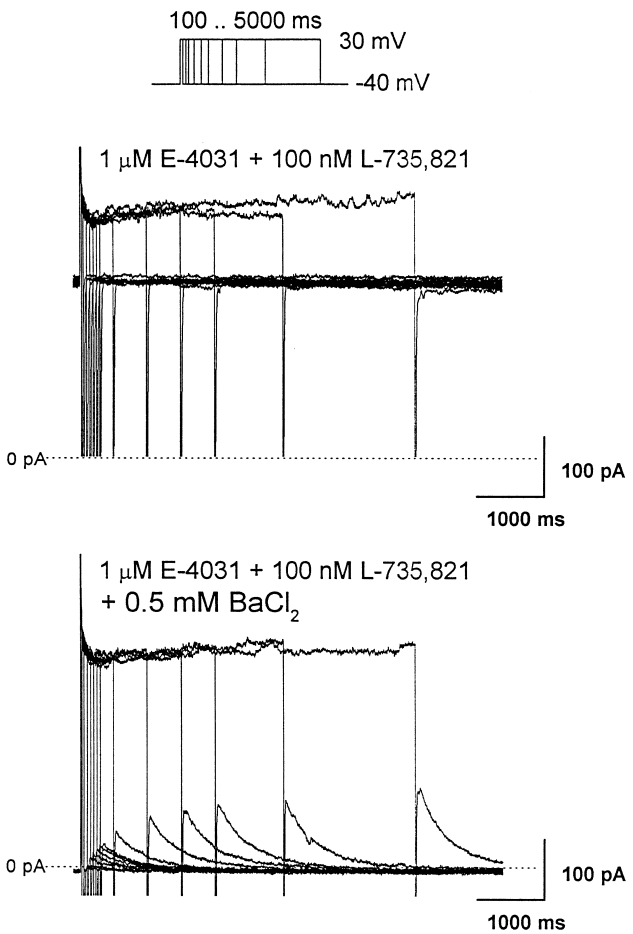

Fig. 6. Ba^{2+} induces a slowly activating outward tail current in a human ventricular myocyte after complete block of both I_{Kr} and I_{Ks}. After superfusion of the myocyte with 1 μM E-4031 and 100 nM L-735,821, no tail current can be noticed (top), but following application of 0.5 mM $BaCl_2$ a slowly developing tail current appears, which can not be attributed to I_{Ks} (bottom).

4. Discussion

The main finding of the present study is that I_{Ks} exists in undiseased human ventricular myocytes and, as in the dog ventricle, exhibits slow activation and relatively rapid deactivation kinetics.

Earlier reports regarding the existence of I_{Ks} in human ventricular myocytes were controversial. In some studies using diseased human ventricles no evidence was found for I_{Ks} [10,17–19]. In our previous study we could identify I_{Kr} but not I_{Ks} in undiseased human ventricular cells [12]. In the present study, we made use of a different pipette solution to that used earlier [12], and it is not yet clear which component of this solution is important for recording I_{Ks}. It is also worth noting that the amplitude of the I_{Ks} tail current reported here is relatively small even in the continuous presence of 1 μM forskolin. In addition, in 27 out of 58 cells I_{Ks} was either absent or was too small to be distinguished from the noise. In an earlier report it was suggested that I_{Ks} might be particularly sensitive to the isolation technique and as such it was difficult to measure it in human preparations [11].

One may speculate that this new pipette solution contained more free K^+ than that used in our previous experiments, and also contained HPO_4^{2-} and ADP, which could enhance intracellular ATP synthesis. In addition, the osmolarity of the new pipette solution was higher than that used previously. This may explain the development of an instantaneous outward current during the time course of our experiments due to the activation of the swelling induced chloride channels. In this study however no attempt was made to clarify the nature of this current.

As mentioned above, in a fairly large number of cells in this study and in the cells in our previous study [12] no discernible I_{Ks} was recorded. It can not be ruled out either that, in addition to the unavoidable slight variation in the cell isolation technique, and the change in pipette solution, the cells might have originated from different layers of the ventricular wall, thereby reflecting regional heterogeneity of the cells rather than merely a technical reason.

As already mentioned, in an earlier study in human ventricular myocytes [11] a relatively large E-4031 insensitive and indapamide sensitive tail current was observed in the presence of Cd^{2+} and Ba^{2+}. This is the only study so far in which I_{Ks} was reported in human ventricular cells. With Ba^{2+} in the extracellular solution, however, we could record an I_{Ks} like tail current in the presence of I_{Ks} and I_{Kr} blockers. Hirano et al. [16] showed that the Ba^{2+} induced I_{K1} block disappeared at positive step potentials, and slowly developed again after returning to

negative voltages and may have appeared as a tail current. We therefore speculate that this tail current could be the result of voltage-dependent interactions of Ba^{2+} with I_{K1} channels. In the study of Li et al. [11], high concentration of indapamide (1 mM) was used to block I_{Ks} and separate it from I_{Kr}. However, indapamide at high concentrations blocks both I_{Ks} and I_{K1} currents [20] and as such it could depress both the I_{Ks} tail current and the I_{K1} current evoked by $BaCl_2$, thereby making interpretation of this study of I_{Ks} uncertain.

Cloned human KvLQT1+MinK channels expressed in COS cells [21] shared some similar properties (activation range, and kinetics) with the current described in this paper. However, the deactivation of the cloned channel seems considerably slower than that of the native current presented in our study. Further data to be obtained under more similar experimental conditions are necessary to understand the reason for the difference between the native and cloned human I_{Ks} currents.

The properties of I_{Ks} measured in the present study most closely resembled those described in dog [8,14] and rabbit [22] ventricular myocytes, i.e. having slow activation and fast deactivation kinetics. The amplitude of I_{Ks} in human seems to be smaller than that reported in dog and rabbit. Whether this is the consequence of the less well-developed cell isolation technique applied in human or reflects different channel densities, remains to be clarified in further studies.

4.1. Possible limitation of the study

This study may have some limitations. The relatively infrequent and irregular access to human heart makes difficult to obtain large number of cells and sometimes to get sufficiently long lasting seals. This is a limitation, which is almost impossible to avoid. It was also previously reported that I_{Ks} is rather sensitive to the isolation technique [11]. Therefore, it can not be ruled out that during the isolation procedure we damaged some of the transmembrane ionic channels particularly I_{Ks}. In spite of these obvious limitations, however, we think that the successive measurements presented in this study provide important evidence about the existence of I_{Ks} and help to elucidate some of its basic properties in undiseased human ventricular cells.

4.2. Potential significance

Our results provide further evidence of the existence of I_{Ks} in the human ventricle. This finding is relevant to previous molecular biology experiments in which expression of the gene encoding this channel was identified [23,24], and mutations were identified that may lead to a disease called long LQT1 syndrome [25]. The exact role of I_{Ks} controlling normal repolarization seems to be uncertain since the current activates slowly and at relatively positive potential compared to the duration and the voltage range of the plateau phase of the action potential. Also, selective blockers of I_{Ks} did not lengthen substantially the normal action potential in the dog papillary muscle, unless other repolarizing currents were affected [14]. Further studies are now needed to elucidate the role of I_{Ks} in the repolarization in the normal human ventricle.

Acknowledgements

This work was supported by grants from the Hungarian National Research Foundation (OTKA T-020604, T-032558), Hungarian Ministry of Health (ETT T-06 125/POT97, 536/2000, T-06 037/1998, 532/2000), Hungarian Ministry of Education (FKFP 1025/1997), the Hungarian Academy of Sciences and by a János Bolyai Research Scholarship (for VL). The authors would like to thank Dr Uwe Gerlach (Hoechst AG, Germany) and Dr Joseph J. Salata (Merck-Sharpe & Dohme, NY, USA) who kindly provided chromanol 293 B and L-735,821. The authors are grateful to Professor G. Dockray for revising the English of the manuscript.

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