ORIGINAL ARTICLE

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Two Components of Voltage Dependent Outward K^+ Current in Isolated Human Atrial Myocytes

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ABSTRACT

Background: The cardiac electrophysiological characteristics differ significantly among mammalian species or among various disease processes. However, difficulties in the procedures for harvesting and isolating tissue have precluded studies using human cardiac specimens. **Methods**: The outward K^+-currents were recorded in human atrial myocytes isolated from patients undergoing open heart surgery. The electrophysiological characteristics of the voltage-dependent outward currents were investigated using a whole-cell patchclamp technique. **Results**: Using depolarizing step pulses, the transient outward currents were activated within 10 msec, which slowly inactivated thereafter. After inactivation, the sustained components of the outward currents remained for up to 5.0 seconds of depolarizing step pulses. While the inactivating component was almost completely inactivated at potentials >+ 30 mV, the non-inactivating component showed only 10−15% inactivation. The non-inactivating component was highly sensitive to 4-AP and was inhibited by >80% at a concentration of 0.2 mM, while the inactivating component was inhibited by only 25%. The delayed rectifier potassium currents were not recorded. The ratios of the amplitudes of the inactivating and non-inactivating components varied. **Conclusions**: Two components of the voltage dependent outward K^+ currents in human cardiac tissue were identified, which could be separated according to their kinetic and pharmacologic properties. **(Korean Circulation J 2004;34(1):92-99)**

KEY WORDS: Human; Atrial appendage; Patch-clamp technique; Potassium channels.

Introduction

It has been widely recognized that there are significant differences in electrophysiological characteristics of the heart among various mammalian species.[1-5] The ionic current present in one species may not be found in another,[1] or the current playing a critical role in one species may not be important in other species.[6] In addition, disease processes can alter the ion channel function, which may subsequently result in changes in the electrophysiological parameters characteristic of the specific disease process.[7-10] Experimental results from animal models can be used to estimate the electrophysiological changes resulting from the pathologic process. However, these experiments have inherent limitations considering the species-specific and disease-specific nature of ion channel remodeling.

Since the procedure for enzymatic isolation of human atrial or ventricular preparations has been established, experiments in human specimens using a patch-clamp technique are being actively performed.[4][5][11][12] However, due to the difficulties obtaining human heart specimens along with isolating single myocytes, a complete understanding of the human cardiac ion channel system is far from being complete.

In order to better understand the electrophysiological characteristics of the human heart, the ionic currents were measured in single atrial myocytes obtained from

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patients undergoing open-heart surgery. Since the repolarization and refractoriness of cardiac tissue is of prime importance in the genesis of cardiac arrhythmia, the ionic current measurements were focused on the repolarizing outward currents, particularly the K^+ currents.

Methods

Isolation of single atrial myocytes

Specimens of the human right atrial appendages were obtained from patients undergoing open heart surgery (Table 1). On excision, the samples were placed in an oxygenated Tyrode solution and brought to the laboratory. The time between excision and beginning of the laboratory process was 10−15 minutes. The procedure for obtaining the tissue was approved by the Ethics Committee of the Seoul National University Hospital.

After transportation, the samples were immersed in a Ca^{2+}-free Tyrode solution (100 % O_2 at 37℃, pH 7.4).

Table 1. Clinical characteristics of the patients

Sex	Age	Diagnosis	Rhythm	Medication
F	24 y	AR	NSR	Yamatetan
M	26 y	MSi	AF	DGX, NCR, RNT
F	56 y	CAD	NSR	ISD, DTZ
M	28 y	MR	NRS	RNT, LSX
M	43 y	CAD	NSR, VPB	ISD, DTZ
F	61 y	ASi	NSR	DGX, RNT
F	49 y	MSR	AF	DGX,HCT
M	3 m	RPA interruption	NSR	None
M	8 y	VSD	NSR	None
F	4 m	VSD	NSR	DGX
M	2 y	VSD	NSR	None
F	55 y	ASi	NSR	RNT
M	67 y	CAD	NSR	ISDN, DTZ
M	63 y	MSi	NSR, VPB	LSX, DGX
M	57 y	CAD	NSR	ISDN, DTZ

AR: aortic regurgitation, MSi: mitrial stenoinsufficiency, MR: mitral regurgitation, CAD: coronary artery disease, RPA: right pulmonary artery, VSD: ventricular septal defect, NSR: normal sinus rhythm, AF: atrial fibrillation, VPB: ventricular premature beat, DGX: digoxin, NCR: nicorandil, ISD: isordil, DTZ: diltiazem, LSX: lasix, RNT: renitec (enalapril), HCT: hydrochlorothiazide, ISDN: isosorbide dinitrate

The cell isolation procedure was developed and modified based on a technique described by Escande et al.[11] The myocardial specimens were chopped with scissors into cubic chunks (< 1 mm^3) and placed in a petri dish containing 10 ml of the Ca^{2+}-free Tyrode solution. The tissue was gently agitated for 5 minutes by continuous bubbling with 100% O_2. After repeating this procedure three times, the chunks were incubated in a similar solution containing 400 IU/mL collagenase (type IV, Sigma) and 4 IU/mL protease (type XXIV, Sigma). The first supernatant was removed after 20 minutes and then discarded. The chunks were reincubated in a fresh enzyme solution containing collagenase only (400 IU/mL). For pediatric specimens, the collagenase and protease levels were reduced to 320 IU/mL and 3.2 IU/mL, respectively. A microscopic examination of the medium was performed every 5 minutes in order to determine the number and quality of the isolated cells. When the yield appeared to reach a maximum, the chunks were suspended in the storage medium.

Only the quiescent rod-shaped cells showing clear cross striations were used for the patch clamp experiments. All the experiments were performed at 35℃.

Voltage-clamp recording and analysis

The membrane currents were recorded in a whole-cell patch configuration using an Axopatch-1C amplifier (Axon Instruments). The electrical signals during the experiments were displayed on an oscilloscope (PM 3350, Phillips, Netherlands) and a chart recorder (Gould). The data was digitized using pClamp software 5.7.1 (Axon instruments) at a sampling rate of 1−2 kHz, and was filtered at 5 kHz. The digitized data was stored using a digital tape recorder (DTR-1200, biologic, France). The patch pipettes were pulled from the borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using a Narishige puller (PP-83, Japan). Pipettes with a resistance of 2−5 MΩ when filled with the pipette solutions were used. Step pulses from −70 mV to +100 mV were applied with a holding potential at −80 mV. The sodium current was inactivated by applying a

25 msec prepulse at −40 mV, and the Ca^{2+}-current was blocked by nicardipine (2.0 mM).

Solutions and chemicals

The normal Tyrode solution contained NaCl 143 mM, KCl 5.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.0 mM, glucose 5.5 mM and HEPES 5.0 mM (pH was adjusted to 7.4 with NaOH).

The storage medium for the isolated myocytes contained KOH 70 mM, L-glutamate 50 mM, KCl 40 mM, KH_2PO_3 20 mM, $MgCl_2$ 3.0 mM, glucose 10 mM, HEPES 10 mM and EGTA 0.5 mM (pH was adjusted to 7.4 with KOH).

The pipette solution contained KCl 140 mM, $MgCl_2$ 1.0 mM, Mg-ATP 5 mM, creatine phosphate-ditris 2.5 mM, creatine phosphate-disodium 2.5 mM, HEPES 10 mM and EGTA 5 mM (the pH was adjusted to 7.3 with KOH).

All the chemicals were purchased from Sigma (St. Louis, MO. USA). The cells were superfused with a solution at a flow rate of approximately 5 ml/min. Approximately 30 seconds were needed to completely change the bath contents.

Statistics and presentation of data

The results in the text and in the figures are presented as a mean±standard error.

Results

Distribution of outward currents in human atrial myocytes

When the 300 ms step pulses were applied from −70 to +100 mV in 10 mV steps with a holding potential at −80 mV, the outward currents were activated rapidly, which then inactivated slowly to various degrees (Figure 1A). After inactivation, the remaining outward currents were maintained at a constant for up to 5 seconds during the application of the test pulses. The rapidly activating currents emerged from test pulses of −30 to −10 mV. A plot of the current-voltage relationship of the maximum current (peak current, I_p) that was activated during the test pulse, and the steady state current (steady state current, I_{ss}) that remained at the end of the test pulse is shown in Figure 1B.

Inactivation of outward currents

In order to examine the steady-state inactivation of the outward current, 3,000 msec test pulses were delivered at +60 mV after 1,000 msec conditioning steps at potentials ranging from −100 to +30 mV (Figure 2A). The inactivation of the outward current was minimal at the conditioning pulses less than −50 mV. As the voltages of the conditioning pulses were increased from −50

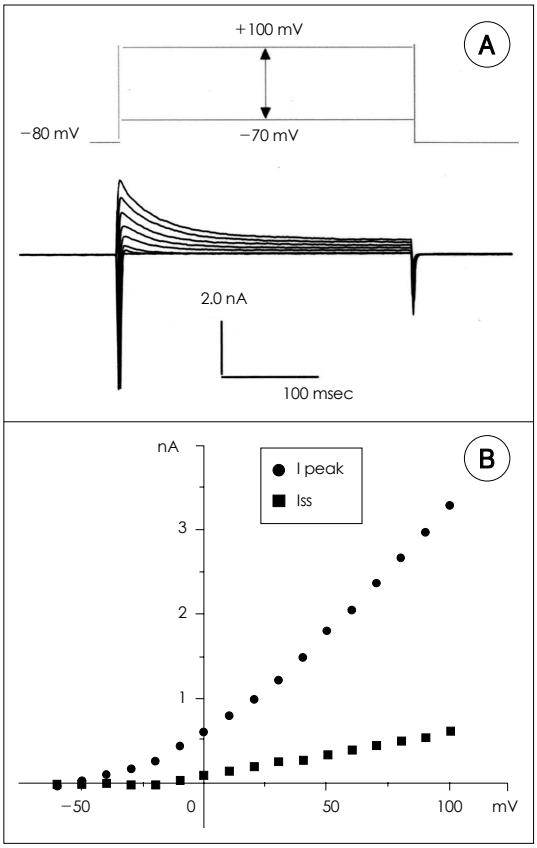

Figure 1. Activation of the outward currents. Superimposed records illustrate the current changes in response to 300 msec depolarizing voltage-clamp pulses from a holding potential of −80 mV. The lower panel is a current-voltage (I−V) curve obtained by measuring these current changes at their peak and steady-state.

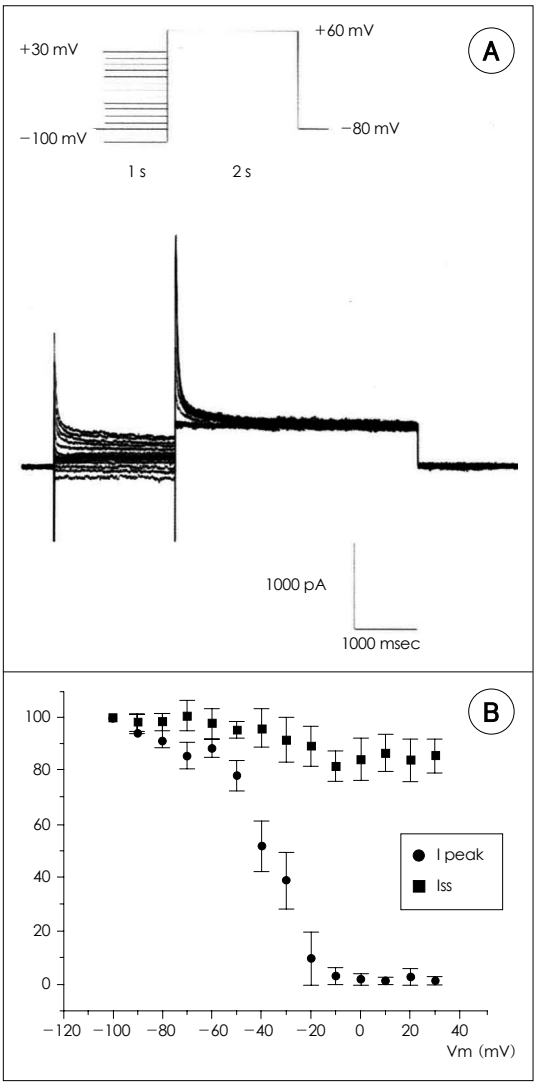

Figure 2. Inactivation of the transient outward current and the ultrarapid delayed rectifier current. In order to study the inactivation of the outward current, 3,000 msec test pulses were delivered at +60 mV after 1,000 msec conditioning clamp steps. The maximum amplitude of the current during the test pulse was normalized to the amplitude of the current following the conditioning step pulses at −100 mV, and were plotted against the membrane potentials of the conditioning pulse. The inactivating (I_p-I_{ss}) component was almost completely inactivated at potentials positive to −20 mV, while the non-inactivating portion of the outward current (I_{ss}) showed only 10 to 15% inactivation throughout the conditioning potentials of −100 to +30 mV.

to −30 mV, the amplitude of the current peak (I_p) decreased rapidly, and at potentials greater than −20 mV, the inactivating components (I_p-I_{ss}) became negligible. In contrast, the amplitude of I_{ss}, which represents the non-inactivating component of the outward current, showed little difference throughout the conditioning potentials between −100 and +30 mV (decreased only by 10 to 15% by conditioning prepulses >0 mV). In order to obtain the steady-state inactivation curves of the inactivating and non-inactivating components of the outward current, the amplitudes of the inactivating components (I_p-I_{ss}) and the non-inactivating components (I_{ss}) during the test pulses (+60 mV) were normalized to their maximum amplitudes following the conditioning step pulses at −100 mV. The values (filled circles for $I_{peak}-I_{ss}$; filled squares for I_{ss}) are plotted as a function of the membrane potentials of the conditioning pulse (Figure 2B).

Kinetic and pharmacologic separation of outward currents

The non-inactivating component was separated from the rapidly inactivating component (I_p-I_{ss}) of the outward currents assuming that the outward current is composed of two different components. As the rapidly inactivating component showed almost complete inactivation at potentials greater than −20 mV, step pulses from −60 mV to +50 mV were used with a holding potential kept at −20 mV. This voltage clamp protocol almost abolished the rapidly inactivating component (the degree of inactivation <5% during the 300 msec step pulses), successfully separating the noninactivating component from the total outward currents (Figure 3).

These two current components were separated using their different sensitivity to 4-aminopyridine. The non-inactivating component, also known as the ultra-rapidly activating K^+ current (I_{Kur}) in previous studies, was named after its very rapid activation characteristics.[5] It has also been shown that I_{Kur} is very sensitive to low dose 4-aminopyridine. In order to determine the concentration-dependent inhibition of the outward current,

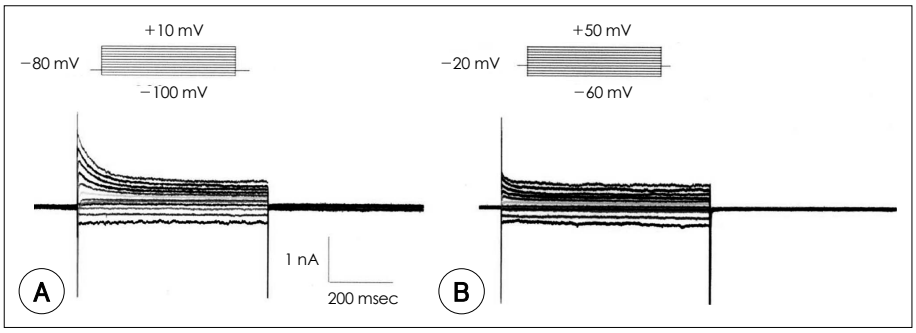

Figure 3. Outward currents recorded by the step pulses with a holding potential kept at −80 mV (A) and −20 mV (B).

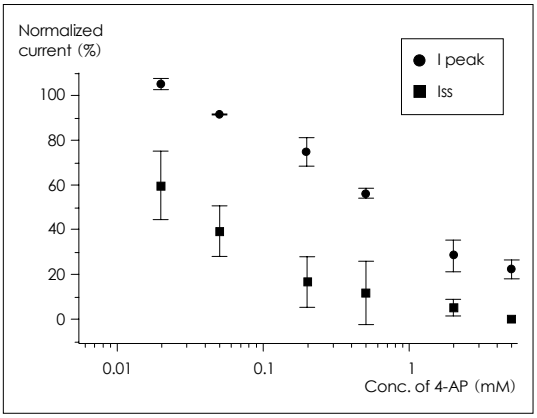

Figure 4. Concentration dependent inhibition of the transient outward current (I_{to}) and the ultrarapid delayed rectifier current (I_{Kur}). In order to determine the sensitivity to 4-aminopyridine (4-AP), the amplitudes of the rapidly inactivating (I_p-I_{ss}) and non-inactivating (I_{ss}) current components were measured at 20 μM, 50 μM, 0.2 mM, 0.5 mM, 2.0 mM, 5.0 mM, and 10.0 mM of 4-AP. The current amplitudes were normalized to the control current (1.0), and plotted against the logarithm of the corresponding 4-AP concentration. The noninactivating component was highly sensitive to 4-AP and was inhibited by >80% at a concentration of 0.2 mM. in contrast, the rapidly inactivating component was inhibited by only ≈25%. At 5.0 mM, the inhibitory effect of 4-AP reached a plateau, and an average of 64% of the total steady-state outward current (I_{ss}) was suppressed.

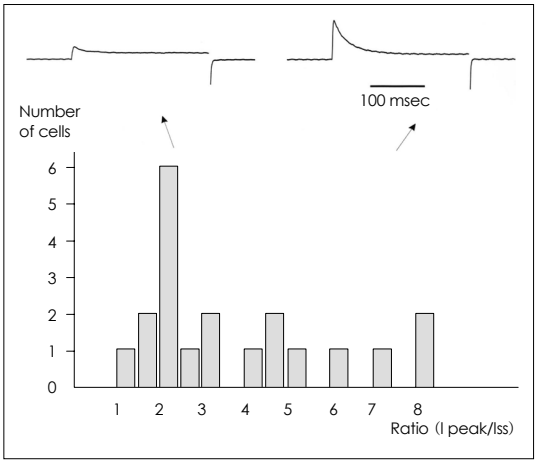

Figure 5. Distribution of the ratios of outward currents. The ratios of the peak (I_p) and the steady state (I_{ss}) outward currents were variable. The outward current was inactivated only slightly in 15% (3/20) of the cells, while in a majority of cells, the outward current inactivated markedly ($2.0<I_p/I_{ss}$ in 85% of cells). This suggests a heterogeneous distribution of ionic currents in human atrial cells.

4-aminopyridine was used at concentrations of 20 μM, 50 μM, 0.2 mM, 0.5 mM, 2.0 mM, 5.0 mM, and 10.0 mM. The amplitudes of the rapidly inactivating (I_p-I_{ss}) and non-inactivating current components measured at each 4-AP concentration were normalized to those of the control current, and the results were plotted as a function of the logarithm of the corresponding 4-AP concentration (Figure 4). The noninactivating component was quite sensitive to 4-AP and was inhibited by >80% at concentration of 0.2 mM 4-AP, while the rapidly inactivating component was inhibited by only ≈25%. At 5.0 mM 4-AP, the inhibitory effect of 4-AP reached a plateau, and an average of 64% of the total sustained outward current was suppressed. The remaining outward current was not further decreased by the addition of either TEA (3 mM), or E-4031 (5 μM) in the superfusate (data not shown), which suggests the

absence of delayed rectifier currents in the present experimental conditions.

Relative contribution of rapidly inactivating and non-inactivating components

The ratios of the peak and the steady state outward currents (I_p/I_{ss}) among 30 cells tested were compared. As shown in Figure 5, the ratios varied widely. The outward current was inactivated only slightly in 15% (3/20) of cells (one example trace in the left panel), while in a majority of cells, the outward current was inactivated markedly ($2.0< I_p/I_{ss} <4.0$ in 45%, and $I_p/I_{ss} >4.0$ in 40% of cells). This suggests the possibility of a heterogeneous distribution of a K^+ channel population in human atrial cells.

Discussion

Isolation of human cardiac myocytes

Experiments using human cardiac tissue have been limited by several factors. The specimens are obtained only during cardiac surgery. The delay in the transport of the specimen to the laboratory and in beginning the enzymatic digestion should be minimized. The isolation of single myocytes was performed by chopping and immersing the tissue in an enzyme solution, not by perfusing through the coronary arteries as in animal experiments.[4)5)11)] Furthermore, this study did not control the age, sex and the disease of the patients, but used all possible specimens. Therefore, errors caused by the variability in the patient factors cannot be excluded in the data interpretations.

The specimens used in these experiments were carried from the operating room to the laboratory within 10−15 minutes. Based on a previously published isolation method and by a minor modification, the cardiac myocytes could be isolated with a clear striation and a high maximum diastolic potential. After 20 minutes incubation in a collagenase and protease solution, the chunks were moved to a collagenase solution. The supernatant was examined periodically every 5 minutes to check the degree of digestion. The optimum number of cells could be obtained within 5−20 minutes of the collagenase treatment. A shorter (<5 min) incubation period resulted in underdigestion, and a longer (>20 min) incubation resulted in the isolation of cells with a poor membrane integrity. The cells could be used for up to 2 days when obtained in the best conditions.

Characteristics of outward potassium currents

The refractoriness of the myocardium plays a major role in the genesis of clinically important cardiac arrhythmias. Excessive prolongation or shortening of the repolarization/refractoriness underlie the development of the Torsades de Pointes or ventricular fibrillation. The process of repolarization is governed by the balance of multiple ionic currents.[13)] The inward currents are carried through the I_{Na}, I_{Ca}, and the Na^+-Ca^{2+} exchange current. A number of K^+ channels, a Cl^- channel and the active Na^+-K^+ pump carry the outward currents. Several distinct outward K^+ currents have been isolated, including the transient outward current (I_{to}), the rapidly and slowly activating delayed rectifier (I_{Kr} and I_{Ks}), the ultrarapidly activating delayed rectifier (I_{Kur}), and the inward rectifier (I_{K1}) K^+ currents. The transient outward current has been reported to be composed of two components.[3)] One component is carried by K^+. This component has a slow decay and is inhibited by 4-AP, but not by the calcium channel blockers. The other component is carried mainly by Cl^-. This component has a shorter rise time and faster decay, and is inhibited by either Ca^{2+} blockers or sarcoplasmic reticulum inhibitors such as caffeine. Because 5 mM EGTA was present in the pipette solutions, the recorded transient outward current in our experiments should consist of the 4-AP sensitive component only.

After inactivating the transient outward current, a large, residual sustained outward current remained. A similar outward current was observed in rabbit or human atrial myocytes. The sustained outward current in the rabbit atrium was characterized as a background Cl^- current.[1)] On the other hand, a similar depolarization-

induced outward current recorded in the human atrial myocytes is known to be carried by K^+. This current showed a very rapid activation (an almost instantaneous jump in the depolarizing pulses) but the inactivation was limited and minimal. It was highly sensitive to low 4-AP concentrations, but was insensitive to tetraethylammonium or Ba^{2+}, differing from the classic I_K.[5] Based on the extremely rapid activation kinetics, this current was named the 'ultrarapid delayed rectifier K^+ current, I_{Kur}'. This newly characterized K^+ current has been shown to be a dominant outward current in the human atrium, but not in the ventricle.[12] The relative contribution of the inactivating transient outward current and sustained I_{Kur} varies greatly among dissociated cells (Figure 5). This may contribute to the inherent heterogeneity in the action potential shape and refractoriness at various regions of the human atrial muscle. Similar results regarding the relationship between the relative magnitude of the outward currents and the resultant changes in action potential morphology have been published.[4] The preferential distribution of I_{Kur} at the atrial myocardium, may provide an important clue in the management of clinically important atrial tachyarrhythmias. The currently used K^+ channel antagnoists exert their effect by blocking the rapidly activating delayed rectifier K^+ current. They have a profound effect on the ventricular myocardium when used in patients with atrial tachyarrhythmias, causing drug-induced Torsades de Pointes. By selectively blocking the outward current at the atrial level, and simultaneously, not affecting the ventricular repolarization, the atrial tachyarrhythmias could be managed without a risk of developing fatal ventricular tachyarrhythmias.

The delayed rectifier K^+ current was not recorded in the present experiments using the specific I_{Kr} blcoker, E-4031. Studies on the presence of delayed rectifier K^+ currents in human cardiac myocytes report varying results. Earlier reports have suggested that the typical delayed rectifier current is small or absent in human ventricular myocytes.[7][12] However, specific I_{Kr} blockers delay the ventricular repolarization and cause the long QT syndrome in humans. Recently, Wang et al. reported evidence of two components of the delayed rectifier K^+ current in human atrial and ventricular myocytes.[4][5][14] They showed that the temperature dependence, and rapid run-down of this specific K^+ current may be responsible for the variable results on the existence of I_K. In their study, a run-down of the current could be minimized by using the electrodes with a tip resistance >4 MΩ.

In conclusion, single human cardiac myocytes were enzymatically dissociated. The outward K^+ currents including the I_{to}, and I_{Kur} were recorded in a whole-cell patch clamp configuration. the I_{to} and I_{Kur} could be separated by their kinetic and pharmacological characteristics. These results will not only provide information on the basic electrophysiological characteristics of the human heart, but can also be applied to the studies of electrophysiological remodeling in cardiac diseases.

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