Identity of a Novel Delayed Rectifier Current From Human Heart With a Cloned K⁺ Channel Current


In human myocardium, the nature of the K⁺ currents mediating repolarization of the action potential is still speculative. Delayed rectifier channels have recently been cloned from human myocardium, but it is unclear whether or not these currents are involved in the termination of the cardiac action potential plateau. In intact human atrial myocytes, we have identified a rapid delayed rectifier K⁺ current with properties and kinetics identical to those expressed by a K⁺ channel clone (fHK) isolated from human heart and stably incorporated into a human cell line for the first time. The myocyte current amplitude was 3.6±0.2 pA/pF (at +20 mV, n=15) and activated with a time constant of 13.1±2 milliseconds at 0 mV (n=15). The half-activation potential (V₅₀) was −6±2.5 mV (n=10) with a slope factor (k) of 8.6±2.2 (n=10). The heterologously expressed fHK current amplitude was 136 pA/pF (at +20 mV, n=9) with an activation time constant of 11.8±4.6 milliseconds at 0 mV; V₅₀ was −4.1±2.4 mV (mean±SEM, n=8); and k was 7.0. The conductance of single fHK channels was 16.9 picosemian in 5 mM bath K⁺. Both native and cloned channel currents inactivated partially during sustained depolarizing pulses. Both currents were blocked by micromolar concentrations of 4-aminopyridine and were relatively insensitive to tetraethylammonium ions and class III antiarrhythmic agents. They had a half blocking concentration (Kₐ₅) for block by 4-aminopyridine of ~50 µM but were relatively resistant to clofilium (Kₐ₅ was 60±13 µM, n=6 for fHK). The strong correspondence between the properties of the two currents provides the first demonstration that a specific K⁺ channel produces a rapid delayed rectifier current in human cardiac tissue. (Circulation Research 1993;73:210-216)

KEY WORDS • potassium channels • delayed rectifier currents • human hearts

The first analysis of delayed rectification in heart by Noble and Tsien¹ identified two currents, Iₛ and Iₐ, with distinctly different kinetics and voltage dependence. Recent pharmacological² and electrophysiological³-⁶ data have revealed multiple, often species-specific, cardiac delayed rectifier currents with varying conductances and kinetic properties. Advances in the cloning of ion channels have simplified the study of the complex mixture of ionic currents present during cardiac action potential repolarization. The isolation and expression of individual channel components has led to the description of delayed rectifier channel currents from rat⁷⁻⁹ and mouse¹⁰ heart and also the description of HK2,¹¹ a rapidly activating delayed rectifier current from adult human ventricular myocardium. No information exists on repolarizing delayed rectifier currents in human heart myocytes, so the significance of cloned channels has remained unclear. We have cloned a rapidly activating delayed rectifier channel, expressed it in a human cell line, and correlated it with novel currents recently identified in human atrium.¹² The similarities between the cloned channel currents and the native current in atrium are striking and establish the importance of cloned channels in the study of human cardiac repolarization.

Materials and Methods
Detection and Expression of K⁺ Channel Clone

A pool of six rat brain K⁺ channel cDNAs was random prime-labeled and used to screen a human fetal heart cDNA library (Clontech catalog No. HL1114a). Six positive clones, isolated from 1×10⁵ recombinant clones screened, were subcloned into the Bluescript II vector (Stratagene Inc, La Jolla, Calif) and found to encode portions of the K⁺ channel cloned isolated from human heart (fHK). The longest (1936-bp) cDNA constituted the majority of fHK with the exception of 60 bp of coding sequence at the 5' end of the gene. The missing 60-bp segment was obtained from a 500-bp overlapping clone isolated by screening the library with oligonucleotides from the 5' end of the fHK cDNA. The complete cDNA for fHK was made by attaching synthetic oligonucleotides coding for the first 60 bp (including a consensus sequence for translation initiation) onto the 5' end of the 1936-bp nearly full-length cDNA clone. The entire coding region was then subcloned into
the mammalian expression vector pRC/CMV for transfection into the human embryonic kidney cell line, HEK-293. fHK-pRC/CMV (1 μg) plasmid was linearized with SalI before transfection to facilitate recombination of the plasmid DNA with the HEK chromosomal DNA. The linearized DNA (1 μg) was mixed with 50 μg lipofectin (Bethesda Research Laboratories) and used to transfect 1×10⁶ HEK cells. Forty-eight hours after transfection, G418 (0.5 mg/mL) was added to the media to select for resistant clones. Antibiotic-resistant clones were selected after 2 weeks of growth and maintained in minimum essential medium, 10% fetal bovine serum, penicillin-streptomycin-fungizone (Bethesda Research Laboratories), and G418 (0.5 mg/mL).

Electrophysiology

Specimens of atrial appendages were obtained from the hearts of 10 patients undergoing aortocoronary bypass surgery, ranging in age from 48 to 72 years old. All patients had normal P waves on electrocardiography, and no patient had a history of supraventricular arrhythmias. The protocol for tissue procurement was approved by the ethics committee of the Montreal Heart Institute. The cell isolation procedure was according to the method of Fermi et al¹³ in 1992, which was based on a technique described by Escande et al.¹⁴ Only quiescent rod-shaped cells showing clear cross striations were used, and they were superfused at 3 mL/min with a Tyrode’s solution containing (mM) NaCl, 126; KCl, 5.4; MgCl₂, 0.8; CaCl₂, 1.0; NaH₂PO₄, 0.33; HEPES, 10, and glucose, 5.5; pH was adjusted to 7.4 with NaOH. The bath temperature was 23° to 25°C for all data presented here. Embryonic kidney cell lines incorporating fHK were maintained at 37°C in a 95% O₂-5% CO₂ incubator in 35-mm Petri dishes on glass coverslips coated with poly-d-lysine. Coverslips were removed from the incubator before the experiments and placed in dishes containing the experimental solution at 23° to 25°C. Whole-cell currents and cell-attached patch-clamp recordings were made using variations of the patch-clamp technique. Electrodyes of 1- to 5-MΩ resistance were pulled from Corning 7052 glass (thin wall, Corning Glass Inc, Corning, NY) on a horizontal Flaming-Brown micropipette puller, fire-polished, and filled with a standard internal solution for whole-cell recording that contained (mM) potassium aspartate, 120; KCl, 20; Na₂-ATP, 4.0; HEPES, 5.0; MgCl₂, 1.0; and EGTA, 10; pH was adjusted to 7.2 with KOH. For cardiac myocytes, a modified internal solution was used that contained (mM) KCl, 130; MgCl₂, 1.0; HEPES, 10; EGTA, 5.0; Mg-ATP, 5.0; and Na₂-creatine phosphate, 5.0; pH was adjusted to 7.2 with KOH. An EPC-7 (List Electronic) or an Axopatch 1D (Axon Instruments, Foster City, Calif) amplifier was used for voltage-clamp measurements; data were filtered at 0 to 10 kHz before digitization via a Labmaster DMA interface. The pCLAMP suite of programs was used for data acquisition and analysis. Analog capacity compensation and 50% to 70% series resistance compensation was used during all whole-cell measurements. Isolated single embryonic kidney cells used for electrophysiological analysis had a mean cell capacitance of 21.8±2.9 pF (mean±SEM, n=20). Capacitance was measured by integration of the uncorrected capacity transient. The external solution contained (mM) NaCl, 130; KCl, 5.0; sodium acetate, 2.8; MgCl₂, 1.0; HEPES, 10; glucose, 10; and CaCl₂, 1.0; pH was adjusted to 7.4 with 1N NaOH. HEK cells possessed a small endogenous delayed rectifier current that rarely exceeded 100 to 200 pA in amplitude at the most positive potentials studied and failed to inactivate during long voltage-clamp depolarizations. The overexpression of fHK at 50 to 100 times the level of endogenous current permitted a clear delineation of fHK current in whole-cell and macropatch measurements (eg, see Fig 3D). For myocytes, special steps were taken to ensure that current recordings were not contaminated by other ionic currents. Recordings were filtered at 1 kHz, series resistance was compensated, and leak current was subtracted. Sodium current was inactivated by holding the membrane potential positive to −50 mV or by isotonic replacement with choline chloride for NaCl. CoCl₂ was always present to block Ca²⁺ and Ca²⁺-activated currents. To minimize contamination from other K⁺ currents in myocytes, 10 mM tetraethylammonium (TEA), 1 mM BaCl₂, and 100 nM atropine were present in the bathing medium. Chemicals were from Sigma Chemical Co, St Louis, Mo. TEA was included in the external bath solution by equimolar replacement of NaCl. Clofibrate was dissolved in dimethyl sulfoxide or Tyrode’s solution to make a 0.1-M stock solution and diluted in saline to form final bath concentrations of 1 to 100 μM.

Results

We isolated fHK, a novel clone from human heart, and its amino acid sequence is compared in Fig 1 with other K⁺ channels expressed in human heart. fHK differs from hPCN1, originally cloned from a human insulinoma,¹⁵ in only three N-terminal amino acid residues. The nucleotide sequences are identical except for these three codons, but at these codons, fHK is identical to HK2 cloned from human ventricle.¹¹ This suggests the possibility of cloning errors in the hPCN1 sequence. Except for two striking differences, fHK is highly homologous to HK2. HK2 lacks a unique stretch of 11 amino acids in the putative extracellular region between domains S1 and S2. Also, fHK has two potential protein kinase A phosphorylation sites in the C terminus, whereas HK2 has only one. Conversely, HK1, also from human ventricle, is quite different from fHK, particularly in the N and C termini. In the standardized nomenclature,¹⁸ fHK, hPCN1, and HK2 might be Kvl5a, b, or c, respectively.

fHK is expressed in both the atrium and ventricle of adult human heart (Fig 2). Reverse transcriptase–polymerase chain reaction using total RNA from adult human atrium and ventricle results in the amplification of a 403-bp fHK fragment. Since one of the polymerase chain reaction primers comes from the unique fHK S1-S2 region, this reaction specifically detects fHK message and not HK2. Because the reverse transcriptase–polymerase chain reaction protocol is not strictly quantitative, we cannot compare the level of expression of fHK in atrium and ventricle other than to say that the gene is expressed at easily detectable levels in both tissues. The presence of fHK transcripts in human atrial tissue was confirmed by the Northern blot shown in Fig 2B.

In view of the lack of information regarding delayed rectifiers in human heart, an electrophysiological comparison was made between fHK stably expressed in a
human embryonic kidney cell line, HEK-293, and K+ currents in myocytes from healthy adult human atrium (Fig 3). Both the atrial current and current from HEK cells expressing fHK showed rapid activation that increased with depolarization. Partial slow inactivation of both currents, especially at more positive potentials, was also noted. The inactivation is not likely to be N type since fHK does not possess the N-terminal ball peptide sequence\(^{16,17}\) (unlike HK1; compare with Fig 1). The striking similarities between native atrial and heterolo-
FIG 2. K⁺ channel clone (fHK) expression in human heart total RNA. Panel A: Reverse transcriptase (RT)–polymerase chain reaction (PCR) detection of fHK sequences in adult human heart total RNA. First-strand cDNA prepared from adult human atrium (A) total RNA (lanes 1 and 2) or adult human ventricle (V) total RNA (lanes 3 and 4) was the template in a PCR using fHK-specific oligonucleotides as primers. Lanes labeled "+RNase" were products of reactions pretreated with RNase to detect the presence of contaminating genomic DNA. Lane 5 shows the expected 403-bp PCR product of this primer pair using cloned fHK cDNA template. Total RNA was prepared from adult human atrium or ventricular muscle (obtained from hypertrophic hearts removed at transplant) with the guanidinium thiocyanate–based RNaGents kit (Promega Corp, Madison, Wis). Total RNA (1 μg) (with or without preincubation in the presence of 20 μg/ml DNase-free RNase [Promega] for 30 minutes at 37°C) was used as template in a first-strand cDNA synthesis reaction (RT-PCR kit, The Perkin-Elmer Corp, Norwalk, Conn) primed with random hexamers. The two oligonucleotides that were chosen as PCR primers allowed the specific amplification of fHK and not HK2 sequences. Primer 1 (5′ gggtgaccgcgcgctct 3′) is from the S1-S2 linker region unique to fHK, and primer 2 (5′ gcgcacgctccagaggu 3′) is downstream in the S5 domain and common to both fHK and HK2. The PCR reactions were done after protocols outlined in the RT-PCR kit using 10 pmol of each primer and 35 cycles: 94°C, 30 seconds; 60°C, 30 seconds; and 72°C, 30 seconds. Ten microliters of each PCR was electrophoresed on a 1% agarose gel and visualized with ethidium bromide. In a control reaction with the fHK plasmid as template, this primer pair resulted in the amplification of a 403-bp fragment, which is the expected size based on the cloned sequence. Panel B: Northern blot analysis of fHK expression in human atrium. Human atrial total RNA (10 μg) was fractionated on a 1% agarose-methylmercury gel and transferred to nitrocellulose. The fHK-pRec/CMV plasmid was linearized with BstEII, and 32P-labeled runoff antisense transcripts corresponding to the 400 bp of coding sequence at the 3′ end of the cDNA were made with SP6 polymerase using the MAXIscript RNA synthesis kit (Ambion, Inc, Austin, Tex). Hybridization was performed at 65°C overnight with rapid hybridization buffer (Amersham Corp, Arlington Heights, Ill) at a probe concentration of 10⁶ cpm/ml. The blot was washed twice for 10 minutes in 2× standard saline citrate and 0.1% sodium dodecyl sulfate at room temperature, followed by two 15-minute washes in 1× standard saline citrate and 0.1% sodium dodecyl sulfate at 65°C. Autoradiography was for 20 hours. Two bands are visible, a major one at 2.5 kb and a less intense one at 1.5 kb. This is the same pattern that was observed when human atrial total RNA was probed with HK2. The mobilities of the RNA size markers are noted on the left.

The characteristics of this novel atrial delayed rectifier are different from the slow currents (Iₖ,₃,19,20 Iₖ,21 minK,22 and the slowly activating delayed rectifier current in heart (Iₖ,₃) and more rapid currents (rapidly activating rectifier current in guinea pig ventricle (Iₖ,₃) classically thought to be important in repolarization in heart. Iₖ,₃ is present in human heart but has a completely different peptide sequence to fHK. When expressed in Xenopus oocytes, Iₖ,₃ activates very slowly without inactivating. Iₖ,₃ exhibits inward rectification rather than the prominent outward rectification of the steady-state current-voltage relations noted here.

The pharmacology of this novel atrial current differs from that of Iₖ,₃ and Iₖ,₃/1ₖ,₃. TEA had virtually no effect on human atrial current (4.8±2.1% reduction at 40 mM, P>0.05) or fHK current (15±4.7% reduction at 100 mM, n=7). Both atrial and fHK currents were much more sensitive to 4-aminopyridine (4-AP) than previously described cardiac K⁺ channels. 4-AP at 50 μM blocked atrial K⁺ current by 51.3±3.2% and fHK current by 46.5±5.4% (n=6). In atrial myocytes, 50 μM 4-AP prolonged the action potential by 66±11%,22 By contrast, Iₖ,₃ is effectively blocked by 10 mM TEA applied externally,23,24 is relatively insensitive to 4-AP,23 and is sensitive to micromolar concentrations of clofilium.25 Atrial myocyte K⁺ channels were comparatively resistant to clofilium. At ±20 mV, 100 μM clofilium reduced current by 41±12% (from 189±21 to 100±16 pA, n=10; holding potential, −80 or −10 mV). fHK current was resistant to clofilium with a half blocking concentration of 60±13 μM (n=6). Interestingly, HK2, which has only limited sequence differences from fHK (Fig 1), has a Kₘ for blocking by clofilium of <1.0 μM.26 Iₖ,₃ was blocked by sotalol, whereas fHK was not affected by concentrations of 100 μM. The 4-AP sensitivity of the present currents may also be compared with that of the rapidly inactivating transient outward current in human atrium, which required 2 mM 4-AP for 49.7±5.1% blockade.12 Thus, the pharmacology of the human atrial and fHK currents clearly sets them in a class apart from other native and cloned cardiac delayed rectifier K⁺ channels and from transient outward currents.
K⁺ channels with single-channel conductances ranging from 5.4 to 16 picosiemens have been suggested to mediate delayed rectification in heart tissue from a number of species, although none were human.⁴⁻⁷⁻⁹ Yue and Marban have described a rapidly activating K⁺ channel (14 picosiemens) similar to the macromolecular rectifiers in rat heart.³⁻⁷⁻⁹ Because of the high expression density of fHK channels in HEK cells, it was difficult to obtain single-channel patches. Patches with multiple channels (2 to 10) could be obtained relatively easily, and transition amplitudes similar to those shown in Fig 4 could be seen. Ensemble currents inactivated during step depolarizations of 0.2 to 1 second in duration. Single-channel patches could sometimes be obtained using high-resistance electrodes (<10 MΩ). During 70-mV depolarizations, such single-channel patches (Fig 4A) revealed openings of fHK channels of approximately 1.2 pA in size. Mean slope conductance for patches from four cells was 16.9 picosiemens (Fig 4D). The ensemble average produced a rapid outward current (Fig 4B) that relaxed slightly toward the end of the pulse, consistent with the kinetics of whole-cell currents.
FIG 4. Cell-attached patch-clamp recordings of human K⁺ channel clone (fHK). The bath contained 5 mM K⁺, and the same solution was used in the recording pipette. Clone J9, a relatively low expressing cell line, was used in an attempt to make membrane patches that contained only a single K⁺ channel. Data were recorded at 4 kHz and digitized at 10 kHz. All recordings in panel A have been leak- and capacity-subtracted and are shown here filtered at 1 kHz. In panel A, sequential sweeps of single-channel activity during 140-millisecond step changes in pipette potential (Vp) from 0 to 70 mV applied 20 milliseconds after the start of each data tracing. Note that the mean resting potential of human embryonic kidney cells expressing fHK was +42 mV so that a Vp of +70 mV correlates with a whole-cell potential of +30 mV. The ensemble current in panel B is the average of 247 sweeps at this pipette potential and shows the macroscopic time course of whole-cell current through these channels. The event amplitude distribution at this potential is shown in panel C for a bin width of 0.05 pA. The peak open probability was 0.6 at this test potential, and the channel opening peak occurred at 1.49 pA. The current-voltage diagram for single-channel currents is shown in panel D. Representative recordings of single-channel openings are shown at potentials of +35 and +50 mV with respect to the cell resting potentials. In the graph shown in panel D, single-channel amplitudes have been plotted against the applied pipette potential for data from four patches and have been fitted by a least-squares linear regression method. The resultant mean slope conductance of the single channels in 5 mM bath K⁺ was 16.9 pS.

Discussion

This study constitutes the first demonstration that a delayed rectifier current in native myocytes from human heart has properties identical with current carried by a cloned K⁺ channel from human heart and expressed in a human cell line. The atrial current can provide important control of repolarization in human heart, and this K⁺ channel must now be considered as a target for antiarrhythmic drug research and therapies. The very rapid activation of this delayed rectifier K⁺ current indicates that it is time to reevaluate the mechanisms of termination of human heart action potentials.

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