Potential Molecular Basis of Different Physiological Properties of the Transient Outward K⁺ Current in Rabbit and Human Atrial Myocytes

Zhiguo Wang, Jianlin Feng, Hong Shi, Amber Pond, Jeanne M. Nerbonne, Stanley Nattel

Abstract—The properties of the transient outward current (Iₒ) differ between rabbit and human atrial myocytes. In particular, rabbit Iₒ is known to recover more slowly than its human counterpart and to show much more frequency dependence. To assess the possibility that these physiological differences may reflect differing expression of K⁺ channel subunit gene products, we used a combination of whole-cell voltage-clamp, heterologous expression, pharmacological, antisense, and Western blot techniques. The inactivation of Iₒ in rabbit atrial myocytes was significantly slowed by hydrogen peroxide, with human Iₒ being unaffected. Use-dependent unblocking with 4-aminopyridine was not seen for rabbit Iₒ nor for Kv1.4 currents in Xenopus oocytes, whereas human Iₒ showed strong use-dependent unblock (as did Kv4 currents). Western blots indicated the presence of Kv4 proteins in both human and rabbit atrial membranes, but Kv1.4 was only detected in the rabbit. Antisense oligodeoxynucleotides directed against Kv4.3, Kv4.2, or Kv1.4 subunit sequences significantly inhibited Iₒ current density in cultured rabbit atrial myocytes, whereas only Kv4.3 antisense significantly inhibited Iₒ in human cells. Neither mismatch oligodeoxynucleotides nor vehicle altered currents in either species. We conclude that, unlike human atrial myocytes, rabbit atrial myocytes express Kv1.4 channel subunits, which likely contribute to a number of important physiological differences in Iₒ properties between the species. To our knowledge, these studies constitute the first demonstration of a functional role for Kv1.4 channels in cardiac membranes and provide insights into the molecular mechanisms of an important cardiac repolarizing current. (Circ Res. 1999;84:551-561.)

Key Words: electrophysiology ▪ cardiac arrhythmia ▪ action potential ▪ antiarrhythmic drug

A rapidly activating and inactivating K⁺ current, known in nerve as A-type current, is widely distributed in various tissues and organ systems of mammals. In cardiac cells, this current has been named the transient outward K⁺ current (Iₒ), and it is believed to play a major role in action potential repolarization.1–6 Iₒ is particularly important in early (phase 1) repolarization and influences the participation of other currents and membrane transport processes by influencing the voltage-time trajectory of the action potential. Changes in Iₒ contribute to action potential changes caused by heart disease;7–11 and Iₒ is a target for many antiarrhythmic drugs.12–16 Because of its important role in cardiac electrical activity, major efforts have been made to understand the biophysical properties and the molecular basis of Iₒ. Recent advances in molecular biology have resulted in the cloning of a large number of distinct cDNAs encoding K⁺ channel subunits. The heterologous expression of a variety of cloned K⁺ channel α-subunits, including Kv1.4,17,18 Kv3.3,19 Kv3.4,20 Kv4.1,21 Kv4.2,22–25 and Kv4.3,26–28 results in rapidly activating and inactivating K⁺ currents with characteristics similar to those of endogenous cardiac Iₒ.

The first cardiac clone found to encode an Iₒ-like current was Kv1.4.17 It was initially believed to be a strong candidate to underlie Iₒ in the heart; however, there are important discrepancies between the kinetics of Kv1.4 inactivation and those of native human Iₒ.17 and the distribution of Kv1.4 protein does not parallel that of Iₒ in the rat.29,30 Subsequent work has pointed to an important role of Shal-type genes, particularly Kv4.2 and Kv4.3, as a molecular basis for mammalian cardiac Iₒ.27,31–33 Thus, it is now widely accepted that Kv4 genes are important in encoding cardiac Iₒ and uncertain whether Kv1.4 participates at all in cardiac Iₒ.

The rabbit heart classically has a very important Iₒ,1 and has served as a widely used model for the analysis of the role of Iₒ in cardiac repolarization. There are important differences between Iₒ in the rabbit and the corresponding current in humans,4 with human Iₒ strongly resembling that of other species, including rat,5 ferret,6,23 and dog.6 A striking difference is the slow reactivation rate of Iₒ in the rabbit1,4 compared with other species. The mechanisms underlying the unusual properties of rabbit Iₒ are unknown. Slow reactiva-
tion is typical of Kv1.4, apparently because of an essential role of C-type inactivation in determining the recovery rate. The physiological differences between human and rabbit $I_o$ could be, at least in part, due to the unique participation of subunits encoded by Kv1.4 in the rabbit heart.

The purpose of the present study was to obtain further information about the potential basis for the differences between rabbit and human atrial $I_o$. A variety of techniques, including whole-cell patch clamp, pharmacological probes, antisense oligonucleotides, and detection of ion channel protein expression in cardiac cell membranes, were used to evaluate the hypothesis that differences in the molecular composition of rabbit and human $I_o$ account for differences in physiological properties.

Materials and Methods

Cell Isolation

Procedures for isolating cells from human tissues and from rabbit hearts were based on approaches described previously in detail. When cells were cultured, all procedures were conducted under strictly sterile conditions. Specimens of human right atrial appendage were obtained from hearts of 26 patients (mean age, 62 years; range, 41 to 78 years) undergoing aortocoronary bypass surgery. The atria were all grossly electrodegraphic P-wave abnormalities. The procedure for obtaining the tissue was approved by the Ethics Committee of the Montreal Heart Institute. Tissue chunks of containing the tissue was approved by the Ethics Committee of the Montreal Heart Institute. Tissue chunks of

TABLE 1. Primer Pairs for Cloning

<table>
<thead>
<tr>
<th>Clones</th>
<th>Primer</th>
<th>Position, bp</th>
<th>Size, bp</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.4</td>
<td>Forward 5'-AACAGTCACATGCTCATTTAGG-3'</td>
<td>335-720</td>
<td>385</td>
<td>59.8</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-TAGTAAACCTCTCCCTCCTC-3'</td>
<td></td>
<td></td>
<td>57.3</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>Forward 5'-ACTCAGGGACGTCTAATTG-3'</td>
<td>663-910</td>
<td>247</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CCATTCTCATACATAAGC-3'</td>
<td></td>
<td></td>
<td>54.4</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>Forward 5'-CCAGAAGAGACAGACATG-3'</td>
<td>1436-1749</td>
<td>313</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-TGCTCACCTCGCGTGGATG-3'</td>
<td></td>
<td></td>
<td>60.9</td>
</tr>
</tbody>
</table>

*Annealing temperature calculated by nearest-neighbor method using Oligo software.

Cloning of cDNA Fragments From Rabbit Heart and Preparation of Antisense Constructs

Because the genes of Kv1.4, Kv4.2, and Kv4.3 had not been identified in rabbit hearts, cloning of cDNA sequences for these constructs was required to design appropriate antisense oligomers. Degenerate primer pairs for polymerase chain reaction (PCR) were designed (Table 1) on the basis of the published cDNA sequences of Kv1.4, Kv4.2, and Kv4.3, targeting regions with minimal homology to other channel sequences but with strong conservation across species. Reverse transcription was performed for first-strand cDNA synthesis from an RNA sample extracted from rabbit heart. PCR was then conducted to amplify the desired cDNA fragments. PCR products were subcloned into PGEM-T Easy vector (Promega) and subjected to sequencing analysis. Antisense oligomers (20 to 21 bp) were designed on the basis of the cloned cDNA sequences. Human constructs were based on previously published sequences, with regions of 100% identity between rabbit and human chosen. The specificity of antisense oligomers was confirmed by comparison with all other sequences in GenBank with the use of the Basic Local Alignment Search Tool (BLAST). As a control, mismatch oligomers were prepared that were identical to each gene-specific antisense sequence with the exception of 4 or 5 mismatch mutations. The oligodeoxynucleotide sequences used are shown in Table 2. Phosphorothioate oligodeoxynucleotides were synthesized commercially by Research Genetics Inc.

Exposure to Oligodeoxynucleotides

Oligodeoxynucleotide treatment was started 24 hours after the onset of cell culture. Three groups of cultured cells were studied in all series of experiments. One group of cells (referred to as the control group) was exposed to the same volume of vehicle (sterile distilled water) used for oligodeoxynucleotide delivery, a second group was exposed to mismatch oligodeoxynucleotides, and a third group was exposed to antisense oligodeoxynucleotides. For each treatment, the growth medium was removed, and the cells were washed twice with serum-free medium and antibiotics. Medium 199 (1 mL) was mixed with lipofectin (4 μmol/L final concentration, Gibco-BRL) and vehicle alone or vehicle containing antisense (0.5 μmol/L) or mismatch oligodeoxynucleotides (0.5 μmol/L) and then incubated at room temperature for 40 minutes before addition to the cells. After
6 hours of incubation at 37°C, the medium was removed and the cells were washed with fresh medium 199. The cells were then incubated in growth medium (including antibiotics and heat-inactivated serum) with vehicle, antisense, or mismatch oligomers (1 μmol/L) but without lipofectin for another 18 hours before patch-clamp experiments were performed.

**Competitive Reverse Transcriptase (RT)–PCR**

**RNA Purification**

Cultured cells were washed twice with PBS solution containing (in g/L) KCl 0.2, KH₂PO₄ 0.2, NaCl 8, and Na₂HPO₄·7H₂O 2.16 and then incubated in TRizol reagent (GIBCO-BRL). Total RNA was extracted by the acidic guanidinium-isothiocyanate method using chloroform and precipitated with isopropanol. Isolated RNA was quantified from absorbance at 260 nm by spectrophotometry, and the purity was confirmed by the ratio of A₂₆₀/A₂₈₀. Integrity of the total RNA was evaluated by ethidium bromide staining of denaturing agarose gels. RNA samples were stored in DEPC-treated double-distilled H₂O at −80°C.

**Primers**

Degenerate primers were designed on the basis of published cDNA sequences of Kv1.4, Kv4.2, and Kv4.3 with oligonucleotide sequences chosen from cDNA regions with minimal homology among clones. cDNA fragments were synthesized by RT-PCR of mRNA from rabbit hearts, and the PCR products were subcloned into pGEM-T easy vector (Promega) for sequencing. The gene-specific primers were then designed on the basis of cloned cDNA fragments for each channel. Specificity of the primer pairs was confirmed by the ratio of A₂₆₀/A₂₈₀. Integrity of the total RNA was evaluated by ethidium bromide staining of denaturing agarose gels. RNA samples were stored in DEPC-treated double-distilled H₂O at −80°C.

**Synthesis of RNA Mimic**

First-strand cDNA was synthesized by reverse transcription and was used as template for subsequent PCR amplification with chimeric primer pairs to obtain a cDNA mimic consisting of a 480-bp fragment of human cardiac α-actin cDNA flanked at the 5′ ends by GSPs, and an 8-nucleotide (GGCCGCGG) linker homologous to the 3′ end sequence of T7 promoter was conjugated to the 5′ end of each forward chimeric primer.

**Reverse Transcription**

RNA mimic samples with serial 10-fold dilutions were prepared and added to a constant quantity of sample RNA (total RNA of 1 μg for each reaction). RNAs were denatured by incubating at 65°C for 15 minutes. Reverse transcription was carried out in a 20-μL reaction mixture containing reaction buffer (in mmol/L) Tris-HCl (pH 8.3) 10, KCl 50, and MgCl₂ 1.5, 1 mmol/L dNTPs (Boehringer Mannheim), 3.2 μg random primer (pdN), (Boehringer Mannheim), 5 mmol/L DTT, 50 units RNase inhibitor (GIBCO-BRL), and 200 units of Moloney murine leukemia virus RT (GIBCO-BRL). First-strand cDNAs were synthesized at 42°C for 60 minutes, and the remaining enzymes were inactivated by heating at 99°C for 5 minutes.

**PCR Amplification**

First-strand cDNA (10 μL) was used as a template for amplification in a total volume of 50 μL of reaction mixture. The reaction mixture contained (in mmol/L) Tris-HCl (pH 8.3) 10, KCl 50, MgCl₂ 1.5, and dNTPs 1; 0.5 μmol/L of each GSP pair; and 2.5 units of Taq polymerase (GIBCO-BRL). Reactions were hot-started at 94°C and continued for 3 minutes for initial melting. The cycling profiles were 30-second denaturing at 94°C, 30-second annealing at 54°C, and 40-second extension at 72°C, for 30 cycles, followed by a final 5-minute period at 72°C.

**Quantification of PCR Products**

Densitometry was used for quantification of PCR products. PCR products were visualized under UV light with the use of a PDI Image System and a Nighthawk camera after ethidium bromide staining of a 1.5% agarose gel. The density of each band was then determined by Quantity One software. A DNA mass marker (100 ng) was run in the gel to analyze the size and quantity of PCR products. The density of the DNA mass ladder was used to generate a standard curve by linear regression with extrapolation to 0 for each experiment. The density of each sample band was then converted to the absolute quantity by calibrating to the standard curve. Based on the principle of competitive PCR, when the target is coamplified with a known amount of internal standard, the logarithmic ratio of target over standard product concentration will be linearly related to the initial amount of standard, intercepting the horizontal axis (target/standard ratio of 1) at an internal standard concentration equal to the concentration of the initial target. Accordingly, plots of log (mimic/target concentration) versus known log (mimic) concentration were generated by linear regression with extrapolation to 0 for each experiment. The density of each sample band was then converted to the absolute quantity by calibrating to the standard curve. Based on the principle of competitive PCR, when the target is coamplified with a known amount of internal standard, the logarithmic ratio of target over standard product concentration will be linearly related to the initial amount of standard, intercepting the horizontal axis (target/standard ratio of 1) at an internal standard concentration equal to the concentration of the initial target. Accordingly, plots of log (mimic/target concentration) versus known log (mimic) concentration were constructed from the data and fit by linear regression to determine the abundance of target message present.

**Whole-Cell Patch-Clamp Recording**

**Electrophysiological Recording From Native Myocytes**

Borosilicate glass microelectrodes (1-mm outer diameter) were used, with tip resistances of 1 to 3 MΩ when filled with a pipette solution containing (in mmol/L) KCl 20, potassium aspartate 110, MgCl₂ 1, HEPES 5, EGTA 5, Mg-ATP 5, GTP 0.1, and Na₆-phosphocreatine 5 (pH 7.3, adjusted with KOH). Cells were superfused with modified Tyrode’s solution at 37°C containing (in mmol/L): NaCl 126, KCl 5.4, CaCl₂ 1, MgCl₂ 1, HEPES 5, and dextrose 10. Cd²⁺ (200 μmol/L) was added to the superfuse to block Iᵥ. Any basal acetylcholine-activated current was inhibited by including atropine (1 μmol/L) in the superfuse. ATP-sensitive K⁺ current (IᵥATP) activation was prevented by including 5 mmol/L ATP in the pipette and 10 μmol/L glyburide in the 

---

**TABLE 2. Oligodeoxynucleotides Used in Antisense Study**

<table>
<thead>
<tr>
<th>Clones</th>
<th>Antisense</th>
<th>Mismatch Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.4</td>
<td>5'-CATAGGCGATGTGGTGTCG-3' (355–335 bp)</td>
<td>5'-CGTAAGCTGTAGCTTACC-3'</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>5'-AATTCAGGCGTCTCTGAGCTC-3' (661–680 bp)</td>
<td>5'-AAGTAGGCTGCTGACCAT-3'</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>5'-CCCATGTCCTCCCTCTGCGG-3' (1455–1436 bp)</td>
<td>5'-CTCATGCGCTACTCTGCTTG-3'</td>
</tr>
</tbody>
</table>

Oligodeoxynucleotides used in this study were all phosphorothioated. Values in parentheses indicate the sequence location of the oligomers.
external solution, \( I_{\text{leak}} \) was inactivated by using a holding potential of −50 mV, by replacing extracellular Na+ with choline, or both.

The electrodes were connected to a patch-clamp amplifier (Axopatch 1-D, Axon Instruments) that applied voltage command pulses with the use of pCLAMP6 software running on an IBM-compatible computer interfaced with a digital/analog converter. Recordings were low-pass filtered at 2 Hz. Junction potential offsets were compensated before formation of the membrane-pipette seal. After gigaseal formation (seal resistance ≥ 20 GΩ), gentle suction was applied to rupture the membrane for whole-cell recording. Cell capacitance averaged 29.6 ± 1.8 and 26.5 ± 1.8 pF before and after compensation, respectively, for human cells, and 49.6 ± 3.2 and 43.5 ± 2.4 pF for rabbit cells. Series resistance averaged 5.2 ± 0.6 and 21.1 ± 0.4 MΩ before and after compensation in human atrial cells and 5.0 ± 0.4 and 1.9 ± 0.6 MΩ in rabbit cells. Leak currents were minimal, and no leakage correction was applied.

The amplitude of \( I_{\text{leak}} \) was measured as the difference between the peak of \( I_{\text{leak}} \) and the current level at the end of the pulse. \( I_{\text{leak}} \) was measured as the amplitude of the current at the end of the test pulse relative to the 0-current level. Individual currents were normalized to the membrane capacity to control for differences in cell size.

Expression and Recording of Currents Encoded by Kv1.4, Kv4.2, and Kv4.3

Kv1.4 was obtained as a kind gift from Dr Arthur Brown (Case Western Reserve University, Cleveland, Ohio). It was subcloned into a modified version of pCR II vector containing a small portion of the 3′-untranslated region of DRK1, including the poly A tail, to allow expression in Xenopus oocytes. Kv4.2 was subcloned into pRK-CMV vector. cRNAs were prepared with the mMESSAGE mMACHINE kit (Ambion) using T7 RNA polymerase after linearization of the plasmid, according to the manufacturer’s protocols. cRNAs were dissolved in DEPC-treated sterile water, stored at −80°C, and diluted immediately before injection. Stage V through VI Xenopus oocytes were injected with 46 nL of cRNA.

Approximately 48 hours after cRNA injection, 2-electrode voltage clamp was performed on individual oocytes. Electrodes were filled with 3 mol/L KCl in agar and had resistances of ~0.5 to 1.0 MΩ when measured in the bath solution containing (in mmol/L) NaCl 100, KCl 5, CaCl2 0.3, MgCl2 2, and HEPES (pH 7.4) 10. The electrodes were connected to a GeneClamp-500 amplifier (Axon Instruments). The pClamp 6 suite of programs was used for data acquisition and analysis. Records were digitized at 5 kHz and filtered at 2 kHz. Experiments were conducted at room temperature (22°C to 24°C).

The entire coding region of Kv4.3 was subcloned into pBK-CMV vector (Stratagene). Kv4.3-pBK-CMV plasmid was linearized with MluI before transfection to facilitate recombination of the plasmid DNA with the HEK chromosomal DNA. HEK cells were incubated in DMEM at 37°C in a CO2 incubator until the cells were 70% to 80% confluent. For each transfection, the cells (1 × 106) were incubated with the linearized DNA (1 μg) mixed with 1 μg of CD8 antigen and 18 μg of the liposome preparation lipofectamine (GIBCO-BRL) for 5 hours at 37°C in serum-free DMEM. Fresh DMEM with 20% FBS was then added to the cells without removing the transfection mixture. Seventy-two hours after the start of transfection, Dynabeads M-450 CD8 (Dynal) were added to the medium at a final bead concentration of 1.4 × 105 beads/mL to permit recognition of the transfected cells. Whole-cell patch-clamp recording was performed on the cells with beads attached (indicating gene expression) 40 minutes after magnetic bead exposure.

Western Blots

Peptides corresponding to unique sequences in the N terminus of Kv1.4 (residues 13 to 37; CSHMPYGYAAQARARERELAHSR) and the C terminus of Kv4.3 (residues 484 to 502; QLEKTTTNEFVDQVFEES) were generated by the Protein Chemistry Laboratory (Washington University Medical Center). The peptides were coupled to the keyhole limpet hemocyanin carrier protein and sent to Caltag (San Francisco, CA) for injection into rabbits. Sera were screened using ELISA, and antibodies were subsequently affinity purified using the ImmunoPure Antigen/Anti-body Immobilization Kit No. 2 (Pierce). ELISA assays on the affinity-purified antibodies revealed that each antibody detected only the peptide against which it was generated.

Rat brain membrane proteins were prepared essentially as previously described. All procedures were performed at 4°C, and all solutions contained a mixture of protease inhibitors (in mmol/L, iodoacetamide 1, 1.10-phenanthroline 1, and pefabloc 0.5 and 1.4 μmol/L pepstatin). Briefly, brains were homogenized in 10 mL of Tris-HCl buffer (5 mmol/L Tris; pH 7.4) with 0.32 mol/L sucrose. After nuclei and debris were pelleted by centrifugation (1000g, 10 minutes), the supernatant was centrifuged at 100,000g for 1 hour. The pellet was resuspended in 20 mmol/L Tris-HCl containing 1 mmol/L EDTA (pH 7.4), centrifuged again (40 000g, 20 minutes), and resuspended in solubilization buffer (20 mmol/L HEPES, 1 mmol/L EDTA, 10% glycerol, 120 mmol/L KCl, and 2% Triton X-100; pH 7.4). After incubation on ice for 1 hour, the final suspension was centrifuged at 78 000g for 2.5 hours to pellet insoluble material. Protein assays (Bio-Rad) were performed, and the samples were aliquoted and frozen at −20°C until use.

Human and rabbit heart membrane proteins were isolated using a protocol similar to that previously described. Rabbit atrial samples were harvested from anesthetized adult rabbits and placed on ice. Adult human atrial tissue samples from normal donor hearts not accepted for transplantation were procured from LifeBanc (Cleveland, OH) with consent for research use. Macroscopic and microscopic examination revealed no evidence of pathology or underlying cardiovascular disease. All procedures were performed at 4°C, and all solutions contained the mixture of protease inhibitors described, as well as 1 mmol/L benzamidine, 7.9 μmol/L aprotinin, and 0.15 μmol/L leupeptin. For the preparation of human membrane proteins, tissue samples (1 to 2 g) from individual donors were processed. Tissue samples were homogenized at 4°C in 10 mL TE buffer (containing 10 mmol/L Tris-HCl and 1 mmol/L EDTA; pH 7.4). After centrifugation (1000g, 10 minutes), the supernatants were retained and the pellets were resuspended to original volume in TE buffer, homogenized, and centrifuged. The supernatants were collected, pooled with the original supernatants, and centrifuged (40 000g, 10 minutes). The resulting pellets were resuspended in TE buffer containing 0.6 mol/L KI, incubated on ice (30 minutes), centrifuged (40 000g, 10 minutes), and then washed twice with TE buffer. The final pellets were resuspended in TE buffer containing 2% Triton X-100 and incubated on ice (1 hour) to solubilize membrane proteins. A final centrifugation (174 000g, 30 minutes) precipitated the insoluble material. After protein assays had been completed, samples were aliquoted and frozen at −20°C for subsequent analysis.

Rat brain membrane proteins and rabbit and human heart membrane proteins (15 to 30 μg) were fractionated by SDS-PAGE and then probed by Western blot analysis with the anti-Kv1.4 and anti-Kv4.3 antibodies. Membranes were fractionated on 10% polyacrylamide gels and transferred to Hybond polyvinylidene difluoride membrane (Amersham). The membranes were washed (3 times in PBS and incubated (1 hour) in blocking buffer III (0.2% l-block blocking buffer [Tropix] in PBS with 0.1% Tween 20), followed by an overnight incubation at 4°C with either the anti-Kv1.4 or the anti-Kv4.3 antibody in blocking buffer. The next day, membranes were washed (10 minutes) and then incubated for 1 hour at room temperature with alkaline phosphatase-conjugated goat anti-rabbit IgG (Tropix) diluted 1:10,000 in blocking buffer III. After incubation, membranes were washed in blocking buffer III for 15 minutes and then for 2 minutes in assay buffer (0.1 mol/L diethanolamine with 1 mmol/L MgCl2, pH 9.8; Tropix). Bound antibodies were detected using Chemiluminescent Substrates for Alkaline Phosphatase (CSPD; Tropix).

Data Analysis

Comparisons among groups were performed by ANOVA with Scheffe’s contrasts. A 2-tailed probability of 5% was taken to indicate statistical significance. Group data are presented as mean ± SEM. Nonlinear curve fitting (the Marquardt procedure) was performed using software for patch-clamp data acquisition and analysis (Clampfit in pClamp6).
negative holding potentials. The kinetic differences between $I_{ox}$ in the 2 species are not due to differences in voltage dependence of inactivation, as shown by the results in Figure 1C. A 1000-ms prepulse from a holding potential of –80 mV was followed by a 200-ms test pulse to +50 mV. In 7 rabbit and 6 human cells, the half-activation potential averaged –33.3±2.1 mV in rabbit and –32.7±2.4 mV in human cells (P=NS), indicating no difference in the voltage dependence of $I_{ox}$ inactivation.

Effects of 4-Aminopyridine (4AP) on $I_{ox}$

4AP is a classical $I_{ox}$ blocker.1–3,15 As illustrated by the data in Figure 2, the responses of human and rabbit $I_{ox}$ to 4AP are quite different. Figure 2A shows $I_{ox}$ elicited in a rabbit cell in the presence of 2 mmol/L 4AP on depolarization for 100 ms from –60 to +40 mV. Results are shown during 2 consecutive pulses (P1 and P2) at 1 Hz after a 1-minute quiescent period (>1 minute). Virtually no time-dependent current is elicited by either the first or second pulse. Figure 2B shows data obtained in a human cell with an identical protocol. During the first pulse, there is a small but measurable $I_{ox}$, and the second pulse elicits a relatively large $I_{ox}$. Figure 2C shows mean data for current in the presence of 4AP relative to control current (4AP/Control) as a function of pulse number during pulsing at 1 Hz after a 1-minute quiescent period in 6 human and 5 rabbit cells. In rabbit cells, strong inhibition is present during all pulses, whereas in human cells there is prominent use-dependent unblocking. Figure 2D shows results obtained with the same protocol applied to Kv1.4 channels expressed in $Xenopus$ oocytes. As was the case for rabbit $I_{ox}$, little 4AP unblocking is observed. In contrast, when Kv4.3 is expressed in HEK cells and the same protocol applied, clear $I_{ox}$ unblocking is seen (Figure 2E). Figure 2F shows mean data for 4AP block obtained in 15 oocytes for Kv1.4, 4 cells for Kv4.3, and 8 oocytes for Kv4.2 and indicates that the absence of use-dependent unblocking of 4AP is typical of Kv1.4 channels.

Effects of H$_2$O$_2$

The inactivation of Kv1.4, but not Kv4.2 or Kv4.3, has been shown to be substantially slowed in response to oxidative stress.27,41,42 We therefore compared the response of rabbit and human $I_{ox}$ to the addition of H$_2$O$_2$ in the superfusate. Typical results are displayed in Figure 3A. Oxidative stress resulting from 0.01% H$_2$O$_2$ dramatically slowed the inactivation of $I_{ox}$ in rabbit myocytes (Figure 3A, left). In contrast, human $I_{ox}$ was not significantly affected by exposure to H$_2$O$_2$ (Figure 3A, right) under the same conditions. Average kinetics before and after H$_2$O$_2$ in 10 rabbit cells and 8 human cells are shown in Figure 3B. Overall, the rapid and slow inactivation time constants of rabbit $I_{ox}$ increased from 21.1±3.0 and 249.2±30.3 ms under control conditions to 34.7±5.1 and 364.8±45.3 ms (P<0.001 for each) after exposure to 0.01% H$_2$O$_2$ in 10 cells. In addition, the proportion of slowly inactivating $I_{ox}$ in rabbit cells was increased by H$_2$O$_2$ from 24±6% to 63±6% of total current (P<0.01). For human $I_{ox}$, the rapid and slow phases of $I_{ox}$ inactivation had time constants of 7.1±1.0 and 22.2±2.3 ms before and 5.1±1.2 and
19.0 ± 2.5 ms (n = 6 cells, P = NS) after exposure to H$_2$O$_2$, respectively. The proportion of slowly inactivating current in human cells averaged 44 ± 16% before and 41 ± 8% after (P = NS) exposure to H$_2$O$_2$.

Detection of Kv1.4 and Kv4.3 Proteins in Rabbit and Human Myocardial Membranes

Western blots of fractionated rat brain membrane proteins with the anti-Kv4.3 antibody revealed a prominent protein band at ∼75 kDa (Figure 4A, lane B). The band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit and human heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.

Western blots of fractionated rat brain membrane proteins with the anti-Kv1.4 antibody revealed a prominent protein band at ∼95 kDa (Figure 4B, lane B), and this band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit and human heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.

Western blots of fractionated rabbit heart membrane proteins with the anti-Kv1.4 antibody revealed a prominent protein band at 95 kDa (Figure 4B, lane B), and this band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.

Western blots of fractionated rat brain membrane proteins with the anti-Kv1.4 antibody revealed a prominent protein band at ∼95 kDa (Figure 4B, lane B), and this band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.

Western blots of fractionated rat brain membrane proteins with the anti-Kv1.4 antibody revealed a prominent protein band at ∼95 kDa (Figure 4B, lane B), and this band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.

Western blots of fractionated rat brain membrane proteins with the anti-Kv1.4 antibody revealed a prominent protein band at ∼95 kDa (Figure 4B, lane B), and this band was eliminated when the antibody was preincubated with the peptide against which it was generated. In Western blots of fractionated rabbit heart membrane proteins, prominent bands at ∼75 kDa were also detected with this antibody (Figure 4A, lanes RA and HA). There is also a broad, intense band at ∼55 to 60 kDa in the blot of the rabbit atrial membrane proteins; this corresponds to rabbit IgG, also detected by the goat anti-rabbit (IgG) secondary antibody. Although no attempt was made to quantify these blots, it is of interest to note that the expression levels of the Kv4.3 protein in rabbit and human heart are similar.
Effects of Antisense Oligodeoxynucleotides on Rabbit and Human $I_{to}$

The above results point to a potentially important role for Kv1.4 in rabbit but not human heart, and the potential participation of Kv4 proteins in both species. This possibility was further assessed by the antisense experiments illustrated in Figures 5 (for rabbit) and 6 (for human cardiomyocytes). Results in rabbit cells exposed to mismatch oligodeoxynucleotides differed from those of rabbit cells exposed to antisense oligodeoxynucleotides directed against Kv1.4, Kv4.2 and Kv4.3 sequences, all of which reduced $I_{to}$ amplitude (Figure 5). In human cells, however, only Kv4.3 antisense produced significant reductions in $I_{to}$ (Figure 6).

Mean data for all cells studied (rabbit, 57, 45, and 59 control cells for Kv1.4, Kv4.2, and Kv4.3, respectively; 34, 29, and 37 cells for Kv1.4, Kv4.2, and Kv4.3 mismatch; and 54, 49, and 57 cells for Kv1.4, Kv4.2, and Kv4.3 antisense; human, 48, 42, and 55 control cells for Kv1.4, Kv4.2, and Kv3.4, respectively; 32, 34, and 41 cells for Kv1.4, Kv4.2, and Kv4.3 mismatch; and 52, 48, and 51 cells for Kv1.4, Kv4.2, and Kv4.3 antisense) are shown in Figure 7. In rabbit myocytes, there was a 45±62% decrease ($P<0.01$, compared with vehicle control) produced by Kv1.4 antisense at $–74$ kDa in all samples blotted with antibody not incubated with antigenic peptide (–). These bands are not seen in the samples blotted with antibody blocked by preincubation with antigenic peptide (+), indicating specificity. Similar results were obtained in experiments with 4 rabbit and 3 human hearts. B, Rabbit and human atrial (RA and HA lanes, respectively) and rat brain (B lanes) membrane proteins (30 μg) were immunoblotted with specific anti-Kv4.3 antibodies and reveal a band at $–74$ kDa in all samples blotted with antibody not incubated with antigenic peptide (–). These bands are not seen in the samples blotted with antibody blocked by preincubation with antigenic peptide (+), indicating specificity. Similar results were obtained in experiments with 4 rabbit and 3 human hearts. B, Rabbit and human atrial (RA and HA lanes, respectively) and rat brain (B lanes) membrane proteins (30, 30, and 15 μg of protein, respectively) were immunoblotted with specific anti-Kv1.4 antibodies. A highly dense band at $–97$ kDa was revealed in brain, and a less intense band of similar molecular mass was detected in rabbit atrium when all samples were immunoblotted with antibody not exposed to antigenic peptide (–). These bands were absent from immunoblots blocked by preincubation of antibody with antigenic peptide (+), indicating specificity. Similar results were obtained from 3 rabbit and 3 human hearts.

Figure 4. Rat brain and human atrial membrane proteins were fractionated by SDS-PAGE and transfected to polyvinylidene difluoride membranes, which were then immunoblotted with subunit-specific antibodies and subsequently developed by chemiluminescence. A, Rabbit and human atrial (RA and HA lanes, respectively) and rat brain (B lanes) membrane proteins (30 μg) were immunoblotted with specific anti-Kv4.3 antibodies and reveal a band at $–74$ kDa in all samples blotted with antibody not incubated with antigenic peptide (–). These bands are not seen in the samples blotted with antibody blocked by preincubation with antigenic peptide (+), indicating specificity. Similar results were obtained in experiments with 4 rabbit and 3 human hearts. B, Rabbit and human atrial (RA and HA lanes, respectively) and rat brain (B lanes) membrane proteins (30, 30, and 15 μg of protein, respectively) were immunoblotted with specific anti-Kv1.4 antibodies. A highly dense band at $–97$ kDa was revealed in brain, and a less intense band of similar molecular mass was detected in rabbit atrium when all samples were immunoblotted with antibody not exposed to antigenic peptide (–). These bands were absent from immunoblots blocked by preincubation of antibody with antigenic peptide (+), indicating specificity. Similar results were obtained from 3 rabbit and 3 human hearts.

Figure 5. Representative experiments showing effects of antisense and mismatch oligodeoxynucleotides on rabbit $I_{to}$. $I_{to}$ was elicited by 700-ms pulses from a holding potential of $–50$ mV to voltages between $–40$ and $+50$ mV with 10-mV increments at 0.1 Hz.

Figure 6. Representative experiments showing effects of antisense and mismatch oligodeoxynucleotides on human $I_{to}$. $I_{to}$ was elicited with 100-ms pulses from a holding potential of $–50$ mV to voltages between $–40$ and $+50$ mV at 10-mV increments and a frequency of 0.1 Hz.
I molecules in the rabbit, nor was show that I antisense effects, we evaluated possible effects on of oligodeoxynucleotides. To verify further the specificity of Kv4.3 antisense produced a significant reduction in expression.

For example, on depolarization to \(-40\) mV, currents were recorded with 100-ms pulses from a holding potential of \(-50\) mV to voltages between \(-40\) and \(+50\) mV (10-mV increments) at 0.1 Hz. \(P<0.05\) and \(*P<0.01\) vs control and mismatch groups.

Kv4.3 antisense produced a significant reduction in \(I_{\text{to}}\) expression.

None of the antisenses significantly altered the kinetics of \(I_{\text{to}}\). For example, on depolarization to \(+40\) mV, the fast and slow inactivation time constants in rabbit cells averaged \(26.4\pm2.3\) and \(261.2\pm40.8\) ms, respectively, under control conditions \((n=31)\), and \(24.3\pm3.9\) and \(246.7\pm38.4\) ms in the presence of Kv4.1 antisense \((n=29)\), \(26.3\pm4.7\) and \(281.5\pm41.1\) ms in the presence of Kv4.2 antisense \((n=25)\), and \(27.2\pm3.4\) and \(282.3\pm42.1\) ms in the presence of Kv4.3 antisense \((n=23)\). In human cells, the inactivation time constants averaged \(7.4\pm1.7\) and \(29.4\pm3.2\) ms under control conditions \((n=20)\) and \(7.3\pm2.0\) and \(27.2\pm3.1\) ms after Kv4.3 antisense \((n=22)\).

The lack of effect of mismatch oligodeoxynucleotides on \(I_{\text{to}}\) in rabbit and human cells argues against nonspecific effects of oligodeoxynucleotides. To verify further the specificity of antisense effects, we evaluated possible effects on \(I_{\text{Kur}}\) in human atrium and \(I_{\text{Ca}}\) in rabbit atrium. The results (Figure 8) show that \(I_{\text{Kur}}\) was not altered by any of the antisense molecules in the rabbit, nor was \(I_{\text{Kur}}\) in human cells affected by Kv4.3 antisense.

Finally, we performed additional experiments to determine the effects of antisense on the concentration of various mRNA species in rabbit atrial myocytes. In each experiment, separate groups of cells from 1 heart were exposed to vehicle, antisense oligodeoxynucleotides against 1 ion channel mRNA species, or mismatch oligodeoxynucleotides. At least 3 experiments were done with each construct. Figure 9A shows typical gels for competitive RT-PCR of Kv4.3 mRNA in cells exposed to vehicle (left), Kv4.3 antisense (middle), and mismatch (right) sequences. The point of identity shifted to the right in the antisense-treated group, indicating a decline in Kv4.3 mRNA expression. Figure 9B illustrates the graphic solution of mRNA concentration with the use of data for Kv4.3. Figures 9C through 9E show that antisense reduced the mRNA concentration of the corresponding species significantly compared with vehicle or mismatch oligodeoxynucleotide–treated cells. To exclude antisense effects on other molecular species, we measured mRNA concentrations of the nontargeted molecular species in response to antisense. No nonspecific effects were seen: with Kv4.1 antisense, Kv4.2 mRNA concentrations were \(6.3\pm1.1\) (control) versus \(6.4\pm1.5\) amol/\(\mu\)g RNA (antisense-treated cells) and Kv4.3 mRNA concentrations were \(12.5\pm2.2\) (control) versus \(12.6\pm2.7\) (antisense) amol/\(\mu\)g RNA; with Kv4.2 antisense, concentrations of Kv4.1 mRNA were \(8.4\pm2.0\) (control) and \(8.2\pm2.6\) (antisense) amol/\(\mu\)g and of Kv4.3 mRNA were \(12.5\pm2.2\) (control) and \(12.1\pm2.4\) (antisense) amol/\(\mu\)g; and with Kv4.3 antisense, mRNA concentrations for Kv4.1 mRNA were \(8.4\pm2.0\) (control) and \(8.2\pm1.2\) (antisense) amol/\(\mu\)g and for Kv4.2 mRNA were \(6.3\pm1.1\) (control) and \(5.5\pm1.8\) (antisense) amol/\(\mu\)g. We were unable to study the effects of antisense on mRNA concentrations in human atrial cells because of the very small number of cells available in the small specimens obtained.

**Discussion**

In the present study, we obtained several lines of evidence suggesting that, whereas Kv1.4 subunits do not appear to play a role in human atrial \(I_{\text{to}}\), they are importantly involved in rabbit cardiac \(I_{\text{to}}\). This conclusion is suggested by the effect of oxidative stress, the lack of use-dependent unblocking of 4AP, and the effect of Kv1.4 antisense on rabbit \(I_{\text{to}}\) with
able in mammalian hearts, 43 but protein expression for
earth. Transcripts for many of these clones are detect-
more, Kv1.4 protein was demonstrated in rabbit heart mem-
I
subunits may not match that of mRNA. 30,44 Direct evidence
for the subunit composition of native I,.

Previous Studies on the Molecular Mechanisms
of I,
A variety of cDNA clones encoding K + channel subunits,
and Kv4.3, 26–28 carry currents resembling I, on heterologous
expression. Transcripts for many of these clones are detect-
able in mammalian hearts, 43 but protein expression for I,
subunits may not match that of mRNA. 30,44 Direct evidence
for the subunit composition of native I, is limited. Studies of
protein expression suggest that members of the Shal (Kv4)
subfamily of K + channel subunits are likely involved in
forming rat, 40 dog, 27 and human 27 I,. Recent studies using
antisense techniques showed that I, in the rat is reduced by
exposure to Kv4.2 or Kv4.3 antisense. 31 The expression of
dominant negative Kv4.2 constructs strongly reduces I,
expression in rats 32 and mice. 33

In the present studies, we found a variety of types of
evidence for differences in the molecular composition be-
tween rabbit and human I,. Rabbit I, showed little use-
dependent unblocking of 4AP, whereas human I, showed
prominent unblocking with repeated depolarizing pulses.
A lack of 4AP unblocking was also found to typify currents
carried by Kv1.4, whereas currents carried by Kv4.2 and 4.3
showed prominent unblocking. Exposure to H2O2 clearly
slowed inactivation of rabbit I, but had no appreciable effect
on human I,. Kv1.4 currents are known to be highly sensitive
to changes in redox potential, whereas those carried by Kv4.2
and Kv4.3 are not. 27,41,42,45 Western blots demonstrated the
presence of Kv1.4 and Kv4.3 protein in rabbit atrial and
ventricular membranes, but only Kv4.3 was found in human
atrium and ventricle. Finally, antisense oligodeoxynucleo-
tides directed against Kv1.4, Kv4.2, and Kv4.3 all reduced I,
currents in cultured rabbit myocytes, but only Kv4.3 anti-

Potential Significance
I, is an important repolarizing current in the heart. Abnor-
malities in I, have been detected in a variety of cardiac
pathologies, including ventricular failure, 56,47 myocardial
infarction, 48 impaired metabolic function, 49 and atrial fibrilla-
tion. 50,51 Furthermore, differences in regional expression of I,
appear to be important in the generation of the T wave, 52 in
explaining the electrophysiological response to myocardial
ischemia, 53 in proarrhythmic reactions to antiarrhythmic
drugs, 54 and in the electrocardiographic response to physio-

tical perturbations such as hypothermia. 55 It is therefore
important to understand the molecular basis of the I, channel.
The present studies are the first of which we are aware to
provide direct evidence for a role of Kv1.4 in forming native
I, channels, in terms of data obtained with Western blot,
pharmacological, and antisense approaches. Preliminary re-

tress have been published that indicate, on the basis of
immunohistochemical and biophysical evidence, that Kv1.4
may encode a portion of I, in ferret left ventricular endocar-
dium. 56 The involvement of Kv1.4 subunits in forming rabbit
I, channels may account for their typically very slow reactiv-

Significant differences have been observed in the reactiva-
tion kinetics of I, among various ventricular regions of the
human heart. 57,58 Whereas subepicardial cells recover rapidly
from inactivation and show little frequency dependence,
much like human atrial cells, subendocardial cells show an
important slow phase of recovery and strong frequency
dependence, much like rabbit atrial myocytes. It is thus quite
conceivable that Kv1.4 channel subunits play a significant
role in contributing to transmural repolarization gradients in
the human ventricle. As mentioned above, preliminary data
suggest this to be the case in ferret ventricle. Differences in
the pharmacological and physiological properties of Kv1.4
and Kv4.3 subunits may have important potential implica-

Figure 9. Downregulation of mRNAs coding for Kv1.4, Kv4.2,
and Kv4.3 by the corresponding antisense deoxynucleotides in
RNA samples extracted from rabbit atrial myocytes. A, Repre-
sentative agarose gels for competitive RT-PCR of Kv4.3 K +
channel subunits in samples from control cells, antisense-
treated cells, and mismatch oligodeoxynucleotide–treated cells.
Lane 0, DNA mass ladder, Lanes 1 through 4, Results obtained
with serial dilutions of RNA internal standard (lane 1, 20 pg; lane
2, 2 p; lane 3, 0.2 pg; and lane 4, 0.02 pg). The lower bands
correspond to channel mRNA; upper bands are internal stan-
dards. B, Graph of the logarithmic ratio of optical intensity of
amplified target DNA/mimic DNA vs logarithm of mimic RNA
intensity. The leftward shift of the intersection of the regression
line with the horizontal axis in antisense-treated cells indicates a
decrease in Kv4.3 mRNA concentration. C, Abundance of Kv4.3
mRNA from groups of myocytes exposed to vehicle (Cont),
Kv4.3 antisense (AS), or mismatch (MAS) (n = 3 analyses per
group, with each analysis performed with atrial cells from 1 indi-
vidual heart; **P < 0.01 vs control). D, Abundance of Kv1.4
mRNA from groups of myocytes exposed to vehicle, Kv1.4 anti-
sense, or mismatch (n = 4 analyses for each group; *P < 0.05 vs
control). E, Abundance of Kv4.2 mRNA from myocytes exposed
to vehicle, Kv4.2 antisense, or mismatch (n = 3 analyses for each
group; **P < 0.05 vs control). C through E, Cont indicates control;
AS, antisense; and MAS, mismatch oligonucleotides.
tions for understanding interspecies and intertissue differences in $I_{to}$ regulation.

Potential Limitations

None of the antisense molecules studied produced complete inhibition of $I_{to}$ in either rabbit or human atrium. This observation is compatible with findings in previous studies of antisense oligodeoxynucleotide effect on K$^+$ channel function. Possible explanations include incomplete inhibition of target subunit protein production by antisense and the existence of as-yet-unidentified subunits contributing to macroscopic currents.

A second issue relates to the nature of the contribution of Kv1.4 subunits to overall current in the rabbit. If Kv1.4 subunits existed in separate, homotetrameric channels from those involving Kv4 subunits, one might have expected to see a change in channel kinetics with antisense exposure and to see use-dependent unblocking of a portion of current (that portion carried by Kv4 channels) in the presence of 4AP. Neither of these phenomena was seen: antisense did not alter kinetics of $I_{to}$ in the rabbit, nor was a component of 4AP unblocking seen. One possible explanation of this observation would be the coassembly of Kv1.4 and Kv4 subunits as heterotetramers. The experiments necessary to determine whether Kv1.4 and Kv4 subunits can coassemble have not, to our knowledge, been performed; however, the observation that a Kv4 dominant negative construct did not inhibit coexpressed Kv1.4 channels makes coassembly unlikely. The final limitation is that our study is limited to $\alpha$-subunits that encode 4AP-sensitive transient outward channel subunits. The potential role of other subunits, including $\beta$ peptides, cannot be assessed from the present experiments.

Acknowledgments

This work was supported by the Medical Research Council of Canada, the Heart and Stroke Foundation of Quebec, the Fonds de la Recherche en Santé du Québec NIH (grant NHLBI RO1-HL 34161), and the Fonds de la Recherche de l’Institut de Cardiologie de Montréal. Dr Wang is a researcher of the Heart and Stroke Foundation of Canada. Dr Pond was supported by a postdoctoral fellowship from NIH. The authors thank Nathalie Talbot and XiaoFan Yang for their excellent technical assistance, Diane Campeau for secretarial help with the manuscript, and Dr David Van Wagoner for providing the human heart samples.

References


Potential Molecular Basis of Different Physiological Properties of the Transient Outward 
K⁺ Current in Rabbit and Human Atrial Myocytes
Zhiguo Wang, Jianlin Feng, Hong Shi, Amber Pond, Jeanne M. Nerbonne and Stanley Nattel

Circ Res. 1999;84:551-561
doi: 10.1161/01.RES.84.5.551
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/84/5/551

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/