Molecular Basis of Transient Outward Potassium Current Downregulation in Human Heart Failure

A Decrease in Kv4.3 mRNA Correlates With a Reduction in Current Density

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Background—Despite advances in medical therapy, congestive heart failure remains a major cause of death in the developed world. A disproportionate number of the deaths of patients with heart failure are sudden and presumed to be arrhythmic. Heart failure in humans and in animal models is associated with prolongation of the action potential duration (APD), the result of downregulation of K⁺ currents—prominently, the Ca²⁺-independent transient outward current (Iₒ). The mechanism for the reduction of Iₒ in heart failure is unknown. The K⁺ channel α-subunit Kv4.3, a homolog of the Drosophila Shal family, is most likely to encode all or part of the native cardiac Iₒ in humans.

Methods and Results—We used ribonuclease protection assays and whole-cell electrophysiological recording to study changes in the level of Kv4.3 mRNA and Iₒ in human tissues and isolated ventricular myocytes, respectively. We found that the level of Kv4.3 mRNA decreased by 30% in failing hearts compared with nonfailing controls. Furthermore, this reduction correlated with the reduction in peak Iₒ density measured in ventricular myocytes isolated from adjacent regions of the heart. There was no significant change in the steady-state level of any other mRNA studied (HERG, Kv1.4, Kir2.1, Kvβ1.3, and the α1C subunit of the Ca²⁺ channel). mRNAs encoding Kv1.2, Kv1.5, and Kv2.1 were found in low abundance in human ventricle.

Conclusions—These data provide further support for the hypothesis that Kv4.3 encodes all or part of the native cardiac Iₒ in humans and that part of the downregulation of this current in heart failure may be transcriptionally regulated.

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Key Words: currents ■ sodium ■ potassium ■ heart failure ■ repolarization

Congestive heart failure is a major cause of death worldwide, with as many as 50% of affected patients dying suddenly. Ventricular tachyarrhythmias are a common cause of sudden death in patients with heart failure; however, the underlying mechanism of these tachyarrhythmias is poorly understood. Action potential prolongation in the absence of other significant electrophysiological changes is a hallmark of failing ventricular myocardium. Prolongation of the action potential, particularly if it is heterogeneous, can predispose to exaggerated dispersion of repolarization and nonexcitable gap reentry. However, action potential prolongation itself is arrhythmogenic; longer action potentials can be associated with repolarization abnormalities such as afterdepolarizations, which can predispose to triggered arrhythmias.

Voltage-dependent K⁺ currents mediate repolarization of cardiac myocytes and hence are critical determinants of the action potential duration. Differential expression of a variety of K⁺ currents may be important in defining regional differences in the action potential profile in the heart. Recently, the cellular and molecular bases of action potential prolongation in failing human myocardium were described. Terminal human heart failure is associated with a functional downregulation of the Ca²⁺-independent transient outward K⁺ current (Iₒ) and inward rectifier K⁺ current (Iᵣ). The mechanism of the functional downregulation is unknown, but it may involve altered transcription, translation, membrane trafficking, subunit assembly, post-translational modification, degradation of channel proteins, or a combination. We sought to determine the mechanism of action potential prolongation in human heart failure by quantifying the level of K⁺ channel gene transcripts in normal and failing myocardium.

There are species-specific differences in voltage-dependent K⁺ channel expression. For example, significant levels of mRNA encoding the rapidly inactivating K⁺ channels Kv1.4, Kv4.2, and Kv4.3 have been observed in rat ventricle.
whereas in canine and human ventricle, there is no detectable Kv4.2, but mRNA encoding Kv1.4, Kv1.5, Kv3.4, and Kv4.3 is present. Based on the biophysical properties and pharmacology of expressed Kv4.3 and its abundance in human ventricle, this gene has emerged as the leading candidate for encoding \( I_{\text{Na}} \) in both dogs and humans. We measured \( I_{\text{Na}} \) in cells isolated from normal and failing human myocardium and performed ribonuclease protection assays (RPAs) on samples from the same hearts excised from regions immediately adjacent to the sections used for cell isolation. The level of Kv4.3 mRNA in ventricular myocardium was compared with \( I_{\text{Na}} \) density in isolated myocytes. To determine whether Kv4.3 encodes all or part of \( I_{\text{Na}} \), we took advantage of the known reduction in current density in myocytes isolated from failing human ventricle, this gene has emerged as the leading candidate for encoding \( I_{\text{Na}} \) in both dogs and humans. The steady-state level of Kv4.3 mRNA in ventricular myocardium was compared with the \( I_{\text{Na}} \) density in isolated myocytes. To determine whether Kv4.3 encodes all or part of \( I_{\text{Na}} \), in the human ventricle, we took advantage of the known reduction in current density in myocytes isolated from failing human ventricles compared with cells isolated from nonfailing hearts. We measured a statistically significant reduction in the level of Kv4.3 mRNA in failing ventricle compared with nonfailing controls. The steady-state level of Kv4.3 mRNA correlated with the density of \( I_{\text{Na}} \) measured in cells isolated from adjacent regions of the myocardium. These data provide further support for the hypothesis that hKv4.3 encodes at least part of the native \( I_{\text{Na}} \) channel and that the reduction in \( I_{\text{Na}} \) in heart failure is consistent with transcriptionally mediated regulation.

### Methods

#### Preparation of cRNA Probes

The templates for preparing the human cRNA probes were generated by subcloning small fragments of the cDNAs encoding ion channel and control genes into pBluescript-SK, pBluescript II-SK (Stratagene), pGEM7, or pSP73 (Promega). The cDNA fragments were isolated by amplification of regions of the full-length cDNA clones using the polymerase chain reaction (PCR), creating appropriate restriction sites for subsequent subcloning. All constructs were confirmed by DNA sequencing. Probe sequences were selected so that no probe had long uninterrupted regions of identity with any mRNA other than the transcript to be tested. There was no evidence for unwanted cross-reaction between any probe and a nonspecific transcript. Protected fragments of the anticipated size confirm specific interaction of the cRNA probe and its target transcript. The hH1 template was designed to protect a fragment in the I-II linker of the Na\(^+\) channel, which exists only in the cardiac isofrom; thus, this is a myocyte-specific cRNA probe.

The standard nomenclature for \( K^+ \) channel genes is used throughout. For each control or target transcript, probes were generated by PCR amplification of a region of the cDNA as described in the text. The table gives the probe, the reference, the numbers of the nucleotide sequence that are protected, and the size of the protected fragment. Each PCR primer pair includes a protected size of 103 base pairs (bp) was used for human cyclophilin (Ambion).

<table>
<thead>
<tr>
<th>Probe</th>
<th>Nucleotides</th>
<th>Size, bp</th>
<th>Primers (F/R)</th>
</tr>
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<tbody>
<tr>
<td>hH1 (Reference 17)</td>
<td>1756–2161</td>
<td>406</td>
<td>(F) CGCGGTTACCGACCTGGTCTCTGAGACGAT</td>
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<tr>
<td>α1C Ca(^{2+}) channel (Reference 18)</td>
<td>5077–5294</td>
<td>218</td>
<td>(F) ATCGGTACCGACCTGGGTTCTGAAGCAGAT</td>
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<tr>
<td>Kir2.1 (Reference 19)</td>
<td>549–742</td>
<td>194</td>
<td>(F) GCCGCTACGGCAAAAGGCAAAAGAAGAAAC</td>
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<tr>
<td>HERG (Reference 20)</td>
<td>2206–2520</td>
<td>315</td>
<td>(F) CGCGGTTACCGACCTGGGTTCTGAAGCAGAT</td>
</tr>
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<td>258</td>
<td>(R) TGAAGCTTTTGGATCCAGGGATATAC</td>
</tr>
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<td>Kv1.4 (Reference 22)</td>
<td>2872–3202</td>
<td>332</td>
<td>(F) AGAGGTACCTAGAGGACAGACACAGCTAA</td>
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<tr>
<td>Kv1.5 (Reference 22)</td>
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<td>289</td>
<td>(F) CATGAGCTTTATTCATCATCATTACCTCAG</td>
</tr>
<tr>
<td>Kv2.1 (Reference 23)</td>
<td>2124–2505</td>
<td>382</td>
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<tr>
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<td>271</td>
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<td>315</td>
<td>(F) TAAGGTACCTAGAGGACAGACACAGCTAA</td>
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isolation and a segment of the lateral wall was used for RNA isolation.

The tissue was quick-frozen in liquid nitrogen within 10 to 15
minutes after tissue harvesting and stored at −80°C until further
processing. Total RNA was prepared either with TRIzol Reagent
(GIBCO BRL) according to the manufacturer’s instructions or with
centrifugation through a CsCl cushion. The integrity of all RNA
samples was confirmed by analysis on a denaturing agarose gel and
quantified by absorbance measurements at 260 nm.

Ribonuclease Protection Assay

RPAs were performed as described previously. All probes
contained regions of plasmid sequence at 1 or both ends of the
transcript, permitting easy distinction between any remaining undi-
gested probe and the shorter, specifically protected region of the
transcript, permitting easy distinction between any remaining undi-
gested probe and the shorter, specifically protected region of the

TABLE 2. Patient Demographics and Medications at Transplantation of Failing Hearts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Institution</th>
<th>Age, y</th>
<th>Sex</th>
<th>Etiology</th>
<th>Medications</th>
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<td>1</td>
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<td>53</td>
<td>M</td>
<td>DCM</td>
<td>Captopril, furosemide, triamterine, digoxin, amiodyarone, vesnarinone, dobutamine, warfarin</td>
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<tr>
<td>2</td>
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<tr>
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<tr>
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<td>M</td>
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<td>Captopril, furosemide, dyrenium, digoxin, KCl, lovastatin, questran, coumadin</td>
</tr>
<tr>
<td>11</td>
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<td>F</td>
<td>ICM</td>
<td>Enalapril, furosemide, digoxin, aspirin, MgCl2, allopurinol, microcide, hydroxyzine, chinoralazepate</td>
</tr>
<tr>
<td>12</td>
<td>JHH</td>
<td>59</td>
<td>F</td>
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<td>Lisinopril, bumetanide, digoxin, clonidine, amidipine, topical nitroglycerin</td>
</tr>
<tr>
<td>13</td>
<td>JHH</td>
<td>59</td>
<td>M</td>
<td>DCM</td>
<td>Enalapril, furosemide, dyrenium, digoxin, amidarone, coumadin</td>
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<tr>
<td>14</td>
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<td>28</td>
<td>F</td>
<td>PPCM</td>
<td>Captopril, furosemide, metolazine, digoxin, KCl, vesnarinone, omeprazole</td>
</tr>
<tr>
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<td>JHH</td>
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<td>M</td>
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<td>Captopril, furosemide, dyrenium, digoxin, amidarone, warfarin</td>
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<td>DCM</td>
<td>Enalapril, furosemide, digoxin, KCl</td>
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</tbody>
</table>

JHH indicates Johns Hopkins Hospital; DCM, dilated cardiomyopathy; UM, University of Munich; ICM, ischemic cardiomyopathy; and
PPCM, postpartum cardiomyopathy.

Isolation of Ventricular Myocytes

and Electrophysiology

Ventricular myocytes were isolated as previously described with
minor modifications. The territory perfused by the LAD from the
tortic root to the apex was excised and perfused via the coronary
artery with a collagenase- and protease-containing Tyrode buffer.
The currents described were measured in cells isolated from the
central third of the myocardial wall, excluding endocardial and
epicardial layers, from the area of the left ventricular anterior free
wall between the LAD and the first diagonal branch. Only Ca2+-
tolerant cells with clear cross striations and without spontaneous
contraction or significant granulation (~20% to 40% of those
isolated in both control and failing hearts) were selected for the
experiments.

The whole-cell configuration of the patch-clamp technique was
used to measure I<sub>Na</sub> at room temperature (22°C to 23°C) in cells
isolated from 4 control and 6 failing ventricles as previously
described. Cell isolation was either not performed or was inade-
Results
RPAs were used to determine the levels of ion channel mRNAs in normal and failing human myocardium. The levels of \( K^{+} \) channel mRNA were correlated with currents measured in myocytes isolated from adjacent segments of tissue. Samples from 7 normal hearts and 17 failing hearts were examined. The average age of the patients from whom the control hearts were obtained was 42 ± 13 years, and that of the transplant recipients from whom the failing hearts were harvested was 52 ± 10 years. Two of the normal and 3 of the failing hearts were from female patients. The cause of heart failure in the transplant recipients was dilated cardiomyopathy in 10 of the patients (59%), ischemic/sofitinfection cardiomyopathy in 6 (35%), and postpartum cardiomyopathy in 1 (6%).

The demographics and drug regimens of the patients in the failing cohort are summarized in Table 2. All of the patients were on standard heart failure medical regimens at the time of transplantation, including diuretics, ACE inhibitors, and digitals; 4 of the patients were taking amiodarone, and no other antiarrhythmic drugs were used in this cohort. The age, sex, and reasons for not transplanting the normal hearts are given in Table 2.

Expression of Kv4.3 mRNA in Heart Failure
We previously reported that the Kv4.3 channel is likely to underlie a significant fraction, if not all, of the \( Ca^{2+} \)-

indipendent \( I_{\text{to}} \)\textsuperscript{15}\). The level of Kv4.3 mRNA in the ventricular myocardium of normal and failing hearts was compared to further test the hypothesis that Kv4.3 is a component of the native \( I_{\text{to}} \) and to determine whether \( I_{\text{to}} \) downregulation in heart failure is correlated with downregulation of Kv4.3 mRNA levels. Figure 1A shows a representative RPA for Kv4.3 mRNA in which samples from normal and failing ventricular myocardium are compared; lane P shows the size of the unprotected probe, and lane t contains rRNA and no protection is observed (5 normal and 7 failing samples are shown). There is sample-to-sample variability in both normal and failing hearts: the range of transcript densities in arbitrary units is 0.79 to 1.34 and 0.36 to 0.81 for normal and failing samples, respectively. Repeated assays on the same samples reveal little intrasample variability; the maximal difference on repeated determinations was 17%, and in general, the densities of the protected fragment for Kv4.3 varied by <8%. Overall, in these 12 samples, there is a 34% reduction in the unnormalized Kv4.3 mRNA in failing myocardium compared with that in the control hearts (Figure 1B, \( P<0.01 \)).

Correlation Between mRNA Levels and Current Density
Myocytes were isolated from an adjacent region of the ventricle for the 12 samples shown in Figure 1A: \( I_{\text{to}} \) current density was recorded in at least 2 cells from 10 of these hearts (normal sample No. 2 and heart failure No. 4 current records were obtained from only 1 cell). Representative current records elicited by a family of depolarizing voltage steps from −30 to +80 mV in increments of 10 mV from a holding potential of −60 mV are shown in Figure 1C. The mean and SD values of the current densities at +40 mV in 25 cells from 7 failing hearts and 16 cells from 5 control hearts are shown in Figure 1D (4.1 ± 2.8 pA/pF for failing and 7.9 ± 3.3 pA/pF for control, \( P=0.0035 \)). The change in Kv4.3 mRNA in failing myocardium is consistent in both direction and magnitude with the change in native \( I_{\text{to}} \) measured in ventricular myocytes isolated from the same hearts. We previously demonstrated that the reduction in \( I_{\text{to}} \) in heart failure is due to a reduced number of functioning channels and therefore is a reasonable index of the level of channel protein.\textsuperscript{6} If Kv4.3 encodes the native channel, we anticipate a correlation between the level of mRNA and the density of \( I_{\text{to}} \). Figure 1E illustrates that such a correlation exists; this is a plot of the unnormalized mRNA density and maximal \( I_{\text{to}} \) density at +40 mV (the circles

### Table 3. Patient Demographics and Medications at Transplantation of Control Hearts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Institution</th>
<th>Age, y</th>
<th>Sex</th>
<th>Cause of Death/Reason Not Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UM</td>
<td>55</td>
<td>M</td>
<td>SAH/atherosclerosis of the right coronary artery by inspection*</td>
</tr>
<tr>
<td>2</td>
<td>UM</td>
<td>57</td>
<td>F</td>
<td>SAH/history of hypertension but no documented left ventricular hypertrophy</td>
</tr>
<tr>
<td>3</td>
<td>UM</td>
<td>25</td>
<td>M</td>
<td>Blunt head trauma/question of cardiac contusion</td>
</tr>
<tr>
<td>4</td>
<td>UM</td>
<td>49</td>
<td>M</td>
<td>Blunt head trauma/atherosclerosis of right coronary artery*</td>
</tr>
<tr>
<td>5</td>
<td>UM</td>
<td>21</td>
<td>F</td>
<td>SAH/NA</td>
</tr>
<tr>
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<td>M</td>
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</tr>
<tr>
<td>7</td>
<td>UM</td>
<td>34</td>
<td>M</td>
<td>SAH/NA</td>
</tr>
</tbody>
</table>

UM indicates University of Munich; SAH, subarachnoid hemorrhage; NA, data are not available.

*No evidence for myocardial infarction and left ventricular size and function normal by echocardiography.
Figure 1. Changes in Kv4.3 mRNA in heart failure. A, Representative RPA with probes designed to protect a 337-bp fragment of human Kv4.3 and a 103-bp fragment of cyclophilin. The first lane (P) contains the probes alone. The lane marked t contains yeast tRNA. Shown is 10 μg of total RNA from 5 normal and 7 failing hearts. B, Bar plot of the unnormalized density of Kv4.3 mRNA in 5 normal and 7 failing left ventricular samples; the values are the average of duplicate determinations. The steady-state level of mRNA encoding Kv4.3 is reduced by 34% in failing hearts compared with controls (P=0.009). C, Representative currents elicited by a series of depolarizing voltage steps from –30 to +40 mV in 10-mV increments from a holding potential of –60 mV. The normal traces are from the heart in lane 4, and the failing traces are from the heart in lane 6 (A). D, Bar plot of the current density measured at a test potential of +40 mV in 16 cells from the 5 normal ventricles and 25 cells from the 7 failing ventricles in A. E, Correlation between Kv4.3 mRNA and I\textsubscript{to} density in ventricular myocytes. In 10 samples, 6 from failing and 4 from normal ventricles, RPAs were performed and currents were measured in at least 2 cells isolated from an adjacent region of the myocardium. The maximal I\textsubscript{to} density is plotted against the unnormalized level of Kv4.3 mRNA in arbitrary units determined on the same RPA. F, A similar plot of the Kv1.4 mRNA density versus I\textsubscript{to} density reveals no correlation.
represent normal hearts, and the squares represent failing ventricles). The plot reveals a significant correlation between Kv4.3 mRNA and the current density \( r = 0.89, P = 0.0005 \).

In contrast, the \( I_{\text{to}} \) density did not correlate with the abundance of other mRNAs. Another gene encoding an inactivating K channel, Kv1.4, showed no correlation with the density of \( I_{\text{to}} \) (Figure 1E; \( r = 0.38, P = 0.28 \)).

Expression of the hH1 Na Channel mRNA

It is possible that the change in Kv4.3 mRNA is due to a generalized depression in mRNA levels in failing hearts or to a more specific decline in the expression of genes encoding ion channels. To test this possibility, we examined the level of mRNA encoding the hH1 Na\(^+\) channel in normal and failing hearts. We designed a probe to the hH1 Na\(^+\) channel I-II linker, which is unique to the cardiac isoform of this channel and therefore myocyte specific. The level of hH1 mRNA does not change in failing hearts (Figure 2). The level of hH1 mRNA in failing samples is 96% of that of control hearts (Figure 2B, \( P > 0.7 \)). Similarly, when the steady-state level of hH1 mRNA is normalized to the density of 28S ribosomal RNA to control for sample loading, there is no difference between control and failing ventricles (data not shown). Thus, the reduction in Kv4.3 mRNA is not the result of a generalized decrease in mRNA.

Housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or cyclophilin are typically used as probes to normalize for the total amount of RNA. However, we observed changes in the mRNA encoding both of these proteins in heart failure. For example, there is a 17% decrease in the level of cyclophilin mRNA, which does not reach statistical significance (Figure 1A, \( P > 0.15 \)). This trend in the cyclophilin mRNA level does produce problems with respect to normalization of the channel transcript levels to cyclophilin. The hH1 mRNA does not change with heart failure (Figure 2A and 2B), which is consistent with the lack of changes in \( I_{\text{to}} \) in human atrial cells\(^2\) and canine ventricular cells from failing hearts\(^3\); thus, hH1 may be a more relevant control probe for K\(^+\) channel target genes. We repeated the Kv4.3 RPA with hH1 as the normalizing control probe in 7 control and 17 failing samples. The level of Kv4.3 mRNA normalized to hH1 is reduced by 30% in failing cells compared with nonfailing controls (Figure 2C and 2D, \( P = 0.0005 \)). Because hH1 mRNA reflects the amount of RNA from cardiac myocytes, the reduction of Kv4.3 mRNA is not simply the result of myocyte dropout; instead, the level of

![Figure 2.](image-url)
Kv4.3 mRNA in ventricular myocytes decreases. The intersample variability of Kv4.3 mRNA levels in the heart failure cohort could not be ascribed to patient age, cause of heart failure, or treatment with amiodarone. Three patients with heart failure had Kv4.3 mRNA levels that overlapped with the range of values in normal hearts: 2 of these patients had dilated cardiomyopathy, and 1 had ischemic cardiomyopathy and none were being treated with amiodarone.

**HERG mRNA in Heart Failure**

The ventricular action potential is prolonged in heart failure; similarly, in the genetically determined cardiac arrhythmia, the long QT syndrome (LQTS), repolarization is prolonged. The chromosome 7–associated LQTS is caused by mutations in the human *ether a go-go*–related gene (*HERG*). To test the possible involvement of the *HERG* gene product in the abnormal repolarization associated with heart failure, we measured mRNA levels of this gene in control and failing hearts (Figure 3). *HERG* RNA is abundantly expressed in the human ventricle. There was no significant change in *HERG* mRNA; the average level of *HERG* mRNA expression in failing hearts was 85±18% of the control value (Figure 3B, P=NS).

There was greater intersample variability of *HERG* mRNA expression in control samples compared with the variability of Kv4.3 and hH1 mRNA levels. The SD for the level of expression of Kv4.3 and hH1 mRNAs in control hearts was 16% and 15% of the mean, respectively. In contrast, the value for *HERG* mRNA was 28%. We could not find any link between age or tissue handling that could account for the variability in *HERG* expression in the control samples. The control samples were all taken from the same region of the left ventricle, excluding regional differences in *HERG* expression as a cause of the variability.

**Expression of Inward Rectifier Channel Genes in Heart Failure**

The inward rectifier K+ current (*I* *K*1) is active in the terminal phases of repolarization and is functionally downregulated in human and canine heart failure. The mRNA encoding *I* *K*1 (Kir2.1) is abundant in human ventricle but does not change in failing hearts compared with controls (Figure 4A and 4B). There is significant sample-to-sample variability in the steady-state mRNA levels encoding *I* *K*1; the SD for the level of expression over all samples was 28%. The level of Kir2.1 mRNA in failing hearts is not different from that in control ventricles (Figure 4B, P=0.56).

**Expression of Other Ion Channel Genes in Heart Failure**

The reduction in Kv4.3 mRNA in failing hearts is in contrast to other mRNAs examined (Figure 5). We evaluated the transcript level of several other channel genes known to be present in ventricular myocardium. There was no change in the level of the Kv1 family gene Kv1.4, and we observed very little Kv1.2 and Kv1.5 in human ventricle. The only member of the Kv2 family of genes expressed in rat heart is Kv2.1, and we detected no Kv2.1 in human ventricle. This result is consistent with the absence of any appreciable ultrarapid delayed rectifier K+ current in human ventricular myocytes. In other mammals, Kv3 gene family members are present in low abundance in the heart and are not likely to be relevant to native ventricular *I* *to*.
The β-subunits are known to modulate the function of K⁺ channel α-subunits, in some cases changing the phenotype of a channel from noninactivating to inactivating. The Kvβ1.3 subunit is known to be present in human heart but does not change in heart failure (Figure 5).

The L-type Ca²⁺ channel is active during the plateau of the action potential. Alterations in the magnitude of this current can change the plateau voltage and duration. The mRNA encoding the α1C subunit of Ca²⁺ channel is abundant in human ventricle but does not change with heart failure (Figure 5).

Discussion

One of the hallmarks of ventricular myocardial failure is prolongation of the cellular action potential duration. In humans and animal models, the action potential profile is changed; often, a reduction in the phase 1 repolarizing notch is observed. The prolongation and change in phase 1 are the result of decrease in the Ca²⁺-independent Iₜₒ density. Experiments in small mammals suggest that the Shal subfamily K channel genes Kv4.2 and Kv4.3 encode the native cardiac Iₜₒ. The K channel, Kv4.3, is the leading candidate gene for Iₜₒ in dogs and humans. In further support of this hypothesis, we observed a reduction in Kv4.3 mRNA in heart failure. Remarkably, the reduction in Kv4.3 mRNA is similar to the observed reduction in Iₜₒ density in cells isolated from failing human ventricles. The level of Kv4.3 mRNA was correlated with the Iₜₒ density of ventricular myocytes isolated from segments of the heart adjacent to the samples used for RPA. These data suggest that in the human heart, Kv4.3 encodes all or part of the Ca²⁺-independent Iₜₒ and that downregulation of this current in heart failure is consistent with altered transcription. However, changes in the steady-state level of mRNA can occur by mechanisms other than altered rates of transcription, and mRNA levels cannot be equated with the level of protein.

The correlation of the mRNA encoding Kv4.3 and the current density suggests that changes in the message levels parallel reduction in the functionally important, sarcolemmal component of the Iₜₒ protein. In both human and animal models of heart failure, there are no significant changes in Iₜₒ kinetics or single-channel current amplitude in cells isolated from the same region of normal and failing ventricles; indeed, nonstationary fluctuation analysis was consistent with a reduction in the number of functioning channels in the cell membrane. The number of functioning ion channels serves as a surrogate for the level of channel protein. Therefore, it is reasonable to suggest that the reduction in mRNA transcript is associated with a reduction in the level of immunoreactive protein. However, it is possible that the level of protein does not change despite a reduction in the steady-state level of mRNA encoding Kv4.3 and reduction in Iₜₒ density, and post-translational modifications occur that reduce channel function without altering its biophysical properties. Alternatively, changes in membrane trafficking may result in a reduction in the amount of channel protein in the sarcolemma without a change in the total cellular protein. It will be necessary to generate specific antibodies to Kv4.3 and measure the protein levels directly to distinguish among these possibilities. If Kv4.3 is a component of a heteromultimeric channel that encodes Iₜₒ, based on these data it appears to be rate limiting in terms of production of functional Iₜₒ channel.
The inward rectifier, I_K1, is functionally downregulated in human and canine heart failure. In contrast to Kv4.3 and similar to other K_1 channel subunits measured by RPA in this study, there is no change in the level of Kir2.1 mRNA in failing ventricles compared with controls. It is possible that the reduction in I_K1 is mediated by a post-translational change that eliminates channel function without changing the steady-state level of RNA. It is also possible that the current reduction is mediated post-transcriptionally and that protein levels are indeed reduced in heart failure. Other Kir2 family members have been cloned from human brain and murine heart and brain, suggesting the changes in the expression of these other genes could underlie the change in I_K1.

The delayed rectifier K channel is important in repolarization in heart in a number of species. In humans, the HERG gene encodes the rapid component of the inward rectifier (I_Kr) and is mutated in the chromosome 7-linked form of the long QT syndrome, a congenital cardiac arrhythmia characterized by disordered ventricular repolarization. When the HERG gene is expressed in oocytes or mammalian cells, its electrophysiology and pharmacology resemble those of I_Kr. Kv1.3 blockers prolong the human ventricular action potential, and recently both the rapid component, I_Kr, and the slow component, I_Ks, of the delayed rectifier current were measured in human cardiac myocytes. The mRNA level of HERG is quite variable in human ventricle, but in transmural sections from control and failing left ventricles, it does not change with heart failure. The large variability might be the result of differences in expression of the mRNA and current regionally, so we cannot exclude the possibility that regional changes in HERG mRNA expression occur with heart failure. Alternatively spliced mRNA species of HERG and other mammalian homologs have been recognized recently; the probe used in this study is common to all of the HERG splice variants.

Further study of the apparent transcriptional regulation of Kv4.3 in human heart failure will require study of the regulatory region of this gene. Is the change in transcript level an epiphenomenon due to the altered neurohumoral environment in heart failure, or do the changes in Kv4.3 transcript levels contribute to the development of heart failure? Can the change in expression of this or other K channel genes be altered pharmacologically? If so, will the change in expression alter the incidence of sudden death due to ventricular arrhythmias in heart failure?

**Study Limitations**

A significant limitation of any study in humans is the lack of control of the conditions of the normal and especially the failing ventricles. The anesthetic agents used at the time of cardiac explantation were similar in patients with control and failing hearts, but many other parameters were significantly different. The cause of heart failure was variable, and the duration and severity of myocardial dysfunction and the
medical therapy at the time of explantation of the failing hearts obviously were not controlled. Our experiments were designed to correlate the level of functional protein (ie, \(I_{\text{Kr}}\) density) in cells isolated from these hearts with mRNA levels in immediately adjacent tissue from the same hearts. A limitation of this design is the necessarily small amount of tissue from which all of the measurements can be made; thus, in this study the amount of immunoreactive Kv4.3 protein in the region adjacent to the section of the ventricle used for cell isolation was not determined. Despite these limitations, it is notable that we were able to measure a statistically significant reduction in the mRNA level encoding a K\textsuperscript{+} channel gene that is likely to play a significant role in repolarization of the human heart. Furthermore, the transcript level of Kv4.3 correlates well with the current density of human heart. Furthermore, the transcript level of Kv4.3 across the ventricular wall and in different areas of the ventricles. Regional changes in the K\textsuperscript{+} currents expressed are likely to be important in the local control of ventricular repolarization. However, a study of this sort is better suited to an animal model in which the degree of heart failure, treatment regimen, tissue harvesting, and cell isolation can be controlled.

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Text References


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