Quantitative Analysis of Potassium Channel mRNA Expression in Atrial and Ventricular Muscle of Rats

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Abstract The expression of 15 different potassium channel genes in rat atrial and ventricular muscle was quantitatively compared by use of an RNase protection assay. Of these genes, only five, Kv1.2, Kv1.4, Kv1.5, Kv2.1, and Kv4.2, were expressed at significant levels in cardiac muscle. In comparisons of atrial and ventricular RNA samples, transcripts from the Kv1.2 and Kv4.2 genes showed the largest differences in relative abundance. There was an approximately twofold decrease in total Kv4 subfamily mRNA expression in atrial muscle relative to ventricular muscle and a 70% increase in total Kv1 subfamily mRNA. Variation of potassium channel mRNA expression within the left ventricular wall was also examined. There was a large gradient of Kv4.2 expression across the ventricular wall, and Kv4.2 expression in epicardial muscle was more than eight times higher than in papillary muscle. Other potassium channel genes were expressed at relatively uniform levels across the ventricular wall. The results suggest that transcriptional regulation makes a significant contribution to the control of potassium channel expression in cardiac muscle and to the variation of the electrophysiological phenotype of myocytes from different regions of the myocardium. (Circ Res. 1994;75:252-260.)

Key Words • K+ channels • cardiac muscle • rat myocytes • mRNA expression

The differences in the action potential waveforms of atrial and ventricular myocardium are a classic example of how cellular electrophysiological phenotype is differentiated to serve the different physiological requirements of tissues with related, but distinct, functions.1,2 The electrophysiological properties of atrial and ventricular myocytes, although fundamentally similar, differ in several ways to conform to the functional requirements of the different chambers of the heart.3 Part of the difference in the action potential waveforms of atrial and ventricular myocytes is due to differences in the expression of voltage-gated potassium channels.4-6 There are also notable differences in the waveforms of action potentials in myocardium isolated from different regions of the left ventricular wall, and these differences can be attributed to changes in the relative level of expression of a transient potassium current in different regions of the left ventricular wall.7

It has been shown that voltage-gated potassium channels play an important role in controlling the rate of repolarization of cardiac action potentials,4,8-10 as they do for action potentials in other excitable tissues. The kinetic properties of potassium currents in rat atrial myocytes are significantly different from those in ventricular myocytes, and these differences can account, in part, for the observed differences in the action potential duration in these two cell types.5,11,12 Differences in the potassium currents of atrial and ventricular myocytes from rabbit heart have also been observed, and potassium channels appear to be important determinants of the action potential duration in atrial and ventricular muscle of this species.4 In the guinea pig there do not appear to be large differences in the voltage-activated potassium channels expressed in atria and ventricle.13 This is due, in part, to the absence in guinea pig myocytes of a transient potassium conductance, which is prominent in most mammalian species, including humans, where it is also important in controlling repolarization of the action potential.14,15

In addition to the long-established differences between atrial and ventricular action potential waveforms, it has more recently become apparent that there is a systematic variation within the ventricular wall in the size and rate of the early phase of action potential repolarization. In several species, including dog, cat, and rabbit, it has been shown that epicardial action potentials have a prominent early phase of repolarization that is largely absent in action potentials recorded from endocardial muscle.7 The ionic basis for this difference appears to be largely attributable to the relative absence of a transient outward current (Ito) in papillary and endocardial muscle.16,17

Most voltage-gated potassium channels, which have the putative six-membrane-spanning domain architecture, are encoded by a large gene family, consisting of at least 14 members.18 Potassium channels appear to be assembled from four subunits, and heteromeric channels can be formed by the assembly of subunits coded by two or more different genes.19-22 There are four known subfamilies of voltage-gated potassium channel genes: Kv1, Kv2, Kv3, and Kv4 (the standard nomenclature for the four subfamilies proposed by Chandy23 will be used throughout this article). The subdivision of the potassium channel gene family into four separate subfamilies is based on sequence similarities,24 and this subdivision

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has been shown to have functional significance because only subunits within each of the subfamilies can combine to form heteromeric channels.\textsuperscript{25} Subunits from different subfamilies do not form heteromultimers.\textsuperscript{25} The potential to form heteromultimers within a subfamily means that the functional properties of potassium channels formed from subunits of a given subfamily will depend on which members of that subfamily are expressed in the same cell but will be largely unaffected by expression of potassium channel genes from other subfamilies.

The analysis presented in the present study has three objectives: (1) to determine how many of the genes in the relatively large potassium channel gene family are expressed in typical excitable cells, such as cardiac myocytes, (2) to determine how large the differences in gene expression between the atria and ventricle are and whether these differences can account for the different electrophysiological properties of potassium currents in these two cell types, and (3) to determine what differences exist in potassium channel expression within the left ventricular wall. To achieve these objectives, the expression of 15 potassium channel genes in atrial and ventricular muscle has been compared quantitatively.

Materials and Methods

Preparation of DNA Templates

DNA templates were prepared by subcloning small (180- to 390-bp) cDNA fragments into pBluescript II SK (Stratagene). The cDNA fragments were isolated in one of two ways; either (1) cDNA fragments were isolated as restriction fragments from previously isolated cDNA clones, or (2) cDNA fragments were prepared by reverse transcription and polymerase chain reaction (PCR) amplification from total cellular RNA isolated from rat brain or PC12 cells. The standard nomenclature for potassium channel genes\textsuperscript{23} will be used throughout. For each template, the following sequences were used as probes (the reference nucleotide sequence is given in brackets and PCR primers are given when used): Kv1 (Kv1.1, nucleotides –177 to 206 [RCK1]\textsuperscript{26}; Kv1.2, nucleotides 1487 to 1743 [RBK2]\textsuperscript{27}; Kv1.3, nucleotides 1420 to 1631 [Kv3]\textsuperscript{28}; Kv1.4, nucleotides 1801 to 2132 [RHK1]\textsuperscript{29}; Kv1.5, nucleotides 1888 to 2117 [Kv1]\textsuperscript{23}; and Kv1.6, nucleotides –148 to 165 [Kv2]\textsuperscript{29}; Kv2 (Kv2.1, nucleotides 1931 to 2295 [dkr1]; forward, GCTCTG-GTTTCTTCTTGTCG; reverse, CACGCCTTAGAG-CACCTGAC; and Kv2.2, nucleotides 2051 to 2321 [dkr2]; forward, ATGTCCTTTGACACTGAC; reverse, CTGAC- CAGTGCTCTGAAACATCA), Kv3 (Kv3.1, nucleotides –6 to 236 [Kv4]\textsuperscript{23}; Kv3.2, nucleotides –79 to 301 [RShIIA]\textsuperscript{30}; Kv3.3, nucleotides 5 to 287 [KShIIId1]\textsuperscript{24}; forward, TCACCTC-TGATGGTCTCGT; reverse, ACCGTGTCACGATCT-TACCG; and Kv3.4, nucleotides 476 to 807 [Raw3]\textsuperscript{23}; forward, TGGACATCTTCCAGACCGGGAG; reverse, GCTG- GTGATATCCCTCCAGGC; Kv4 (Kv4.1, nucleotides 279 to 634 [mShall]\textsuperscript{31}; forward, CGTCGTAACCTTT-TATGCCA; reverse, CACGACATGGGATGTCCT; and Kv4.2, nucleotides 309 to 617 [RShall]\textsuperscript{31}, and IsK (IsK, nucleotides 102 to 279 [lK]\textsuperscript{39}).

All constructs were confirmed by sequencing. In the case of genes that are known to produce multiple transcripts, such as members of the Kv3 family, probes to common regions of the transcripts were used. In most cases, probes that hybridized to poorly conserved regions of the coding sequence were used. Probe sequences were selected so that no probe had long uninterrupted regions of identity with any transcript other than the transcript to be tested. Because of the inherent specificity of the RNAse protection assay, there was no evidence for unwanted cross-reaction between any probe and a nonspecific potassium channel transcript.

Preparation of RNA From Atrial and Ventricular Muscle

Atrial and Ventricular Muscle

All RNA samples were prepared from young adult female Sprague-Dawley rats (≈200 g). Each RNA sample was produced from pooled dissections from five animals. For the preparation of atrial muscle RNA, only the left and right atrial appendages were dissected from the heart. In mammals, the parasympathetic cardiac ganglia are concentrated in the interatrial septum, in the superior dorsal surface of the atria, and around the entry points of the major vessels.\textsuperscript{39,40} These regions of atrial muscle were not used. Few, if any, neurons are found in the atrial appendage.\textsuperscript{39} For preparation of RNA from ventricle, only the lower quarter of left ventricular muscle, from the apex of the heart, was used. The ventricular dissection included no papillary muscle and very little endocardium and consisted largely of epicardial muscle, with a smaller contribution from the midlayer regions of the myocardium. Tissue samples were first carefully blotted on paper towels to remove excess blood and then homogenized in guanidinium thiocyanate. Total RNA was prepared by pelleting over a CsCl step gradient. The integrity of all RNA samples was confirmed by analysis on a denaturing agarose gel.

Left Ventricular Dissections

The following procedure was used for experiments on potassium channel expression within the left ventricular wall. The left wall of the left ventricle was dissected free from the rest of the heart and laid out as a sheet on a paper towel. The two largest papillary muscles were then dissected from the bulk of the wall. From the remaining sheet of ventricular wall, a longitudinal strip of muscle was isolated. This strip of ventricular wall was dissected into three layers of approximately equal depth by using forceps and fine irisectomy scissors. The inner endocardial layer was first dissected out, and then the remaining sheet was split in two to give the midlayer and epicardial regions. Samples were prepared from young adult female Sprague-Dawley rats (≈200 g), and each RNA sample was produced from pooled dissections from five animals.

RNase Protection Assay

RNase protection assays were performed essentially as described by Krieg and Melton.\textsuperscript{41} RNA samples were assayed as pairs, with each pair of atrial and ventricular RNA samples coming from the same set of animals. The data were obtained from eight independent sets of RNA samples for the atrial and ventricular comparisons and from three independent samples for the study on variation within the ventricular wall. For each sample point, 5 or 10 μg of total RNA was used in the assay. For the positive control brain sample, 2.5 or 5 μg of total RNA was used. In experiments in which the aim was to compare the level of expression of two different potassium channels, the RNA probes were made by using two DNA templates in the same reaction mixture to minimize differences in the specific activity of the two probes. For comparisons between probes, the data in Table 2 were corrected for differences in specific activity of the probes that were due to different numbers of UTPs in the sequence by assuming that the specific activity was directly proportional to the number of UTPs in each sequence. The small differences in the ratios of RNA expression between atria and ventricle in Tables 1 and 2 are due to the fact that the data analyzed in Table 2 were a subset of the data in Table 1 and contained fewer replications. These differences are not statistically significant. A probe for the rat cyclophilin gene\textsuperscript{42} was included in the hybridization as an internal control. The cyclophilin probe had a specific activity approximately seven-
The atrial-to-ventricular ratios for potassium channel genes expressed at moderate abundance are shown in the first column. The relative abundance of genes whose expression was too low to accurately quantitate is shown in the next column. A —ve score means that no specific signal could be detected in any RNA sample; v.low, on very long exposures (7 to 10 days) a very weak signal could be detected; and low, the abundance of the gene was on the threshold for accurate quantification, but the abundance relative to other members of the subfamily was low.

*P < .01 and †P > .05 (the atrial-to-ventricular ratios were statistically compared with unity by a nonparametric one-sample sign test).

Results

The level of voltage-activated potassium channel gene expression in atrial and ventricular muscle was measured by an RNase protection assay. Considerable care was taken to use rats of similar age and size, because in preliminary experiments it was found that greater variability was introduced when rats of different ages were compared. The 200-g rats were chosen for analysis because they were similar in size to those used in other laboratories for electrophysiological experiments, thereby facilitating comparison between results.

Comparison of Potassium Channel Expression Between Atria and Ventricle

We initially compared potassium channel expression levels between atrial and ventricular muscle by calculating a ratio for the level of expression in atria divided by expression in ventricle.

Kv1 Gene Family

Three of the six genes in the Kv1 family are expressed in rat heart at moderate levels (Fig 1). Two of these genes, Kv1.2 and Kv1.4, are expressed at significantly different levels in comparisons between atria and ventricle (Table 1). Kv1.2 is 2.3-fold higher in atria than in ventricle, and Kv1.4 is 1.5-fold higher. Every sample pair that was tested gave the same result; i.e., they all had higher levels in atria than in ventricle. Kv1.5 was not expressed at significantly different levels in comparisons between the two tissues. For Kv1.5, there was some variability in the results, with expression being higher in either atria or ventricle, depending on which RNA sample was tested.

Expression of the Kv1.3 gene could be detected after 7-day exposures (Fig 1C). The level of expression was low, = 5% of the level of expression of the Kv1.2 gene in ventricle (Table 1). Although it was difficult to measure accurately, scans of long exposures suggested that there was very little difference in Kv1.3 expression between atria and ventricle. In previous studies using either Northern blot analysis or PCR amplification, it was found that Kv1.3 is not expressed in heart. The difference between the present results and previous results can be partly explained by the high sensitivity of the RNase protection assay.

The Kv1.1 and Kv1.6 genes did not appear to be expressed in heart. Even after 7- to 10-day exposures of the gel to x-ray film, we could not detect a specific signal for either mRNA. Positive control brain samples were strongly positive for both mRNAs after an overnight exposure. It has previously been reported that Kv1.1 can be detected in rat atria with Northern blot analysis, although it is possible that this result was due to contamination by neural tissue.

Kv2 Gene Family

Of the two known genes in the Kv2 family, only one, Kv2.1, is expressed in rat heart (Fig 2A). The Kv2.1 gene is expressed at 30% lower levels in atria compared with ventricle (Table 1). This small difference was seen consistently, and in every sample pair that was tested, expression was found to be lower in atria than in ventricle. Similar results for the Kv2.1 gene have been obtained previously. Expression of the Kv2.2 gene could not be detected in heart after a 7- to 10-day exposure, even though the positive control brain sample gave a positive signal after only an overnight exposure (Fig 2A). It has previously been suggested that Kv2.2 is expressed strongly in brain but not in heart.48
been reported that Kv2.2 is expressed just above threshold in ventricle but not expressed in atria.\(^{31}\) We did not find any specific Kv2.2 signal in ventricular samples.

**Kv3 Gene Family**

All of the genes in this family, Kv3.1, Kv3.2, Kv3.3, and Kv3.4, were expressed in rat heart at levels just above the detection threshold. In every case, exposure times of over a week were required to detect even a faint signal, and sometimes a signal was seen in only one of the samples. By contrast, specific signals were easily detected in the brain RNA samples after overnight exposures (Fig 3). In previous studies by other laboratories using Northern blots, the Kv3.1, Kv3.2, and Kv3.4 genes have been found not to be expressed in rat heart.\(^{32,48}\) Expression of the Kv3.3 gene has been described in mouse heart by use of PCR amplification of reverse-transcribed cDNA.\(^{49}\) Although we could detect a very weak signal for all these genes in rat heart, it is probable that this expression level is too low to be of any functional significance. Since the RNase protection assay is capable of detecting down to, or below, the level of one mRNA molecule per cell,\(^{41}\) mRNAs that are expressed at the threshold of detection are very rare. In this situation, either the level of expression is so low as to be insignificant, or the gene is only expressed in a minor subpopulation of cells. In either case, products of the Kv3 gene family probably do not make an important contribution to the electrophysiological properties of the bulk of myocytes.

**Kv4 Gene Family**

Both members of this subfamily are expressed in rat heart (Fig 2B). The Kv4.2 transcripts were considerably more abundant than the Kv4.1 transcripts. The Kv4.2 gene was expressed at significantly higher levels in ventricle than in atria (Table 1) and was expressed at the highest level in the ventricular samples, relative to atrial samples, of any of the potassium channel genes tested. The Kv4.1 gene was expressed at quite low levels and was difficult to accurately measure. The Kv4.1 transcript had a pattern of expression opposite that of the Kv4.2 transcript and was approximately twofold higher in atria than in ventricle. Even in atria, however, the Kv4.1 transcripts constituted a small percentage of the total Kv4 transcripts.

**Relative Levels of Potassium Channel Expression in Atria and Ventricle**

Because the different subunits of the potassium channel subfamilies can potentially form heteromultimers in vivo, it is the relative level of expression of the different subunits that will determine the overall properties of that class of channels. The relative level of gene expression was compared within a gene family where more than one gene was expressed.

**Kv1 Gene Family**

To determine the relative level of mRNA expression, two probes were included in a single hybridization with the RNA samples, and one of these probes was always the Kv1.2 probe (Fig 1). The amount of Kv1.2 transcript in ventricle was arbitrarily given a value of 100, and the relative abundance of the Kv1.3, Kv1.4, and Kv1.5 mRNAs was expressed relative to this value (Table 2). In ventricle, the values for the Kv1.4 and Kv1.5 mRNAs were 43±5 and 104±35, respectively, relative to the level of the Kv1.2 mRNA (Table 2). The Kv1.2 and Kv1.5 genes contributed approximately equal percentages of the total Kv1 subfamily transcripts, with the Kv1.4 gene contributing a lower percentage. The Kv1.3 gene contributed <2% of the total Kv1 subfamily mRNA in ventricle. In atria, the relative percentage of the Kv1.2 transcript in-

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**Table 2. Relative Level of Potassium Channel Expression in Rat Heart**

<table>
<thead>
<tr>
<th></th>
<th>Relative Level of Expression in Atria</th>
<th>Relative Level of Expression in Ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>%</td>
</tr>
<tr>
<td>Kv1 subfamily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kv1.2</td>
<td>234±53</td>
<td>54</td>
</tr>
<tr>
<td>Kv1.3</td>
<td>=5</td>
<td>2</td>
</tr>
<tr>
<td>Kv1.4</td>
<td>73±5</td>
<td>17</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>129±19</td>
<td>29</td>
</tr>
<tr>
<td>Total Kv1-subfamily</td>
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<td>...</td>
</tr>
<tr>
<td>Kv4 subfamily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kv4.1</td>
<td>4±1</td>
<td>7</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>50±11</td>
<td>93</td>
</tr>
<tr>
<td>Total Kv4-subfamily</td>
<td>53</td>
<td>...</td>
</tr>
</tbody>
</table>

The data are scaled relative to Kv1.2 expression in ventricle for the Kv1 subfamily and relative to Kv4.2 expression in ventricle for the Kv4 subfamily. The percent contribution of each transcript is expressed as a fraction of the total transcripts from each subfamily in a given tissue sample. The total values for the Kv1 and Kv4 transcripts are also included for comparison and are scaled to give a value of 100 for the ventricular samples.
increased, the Kv1.5 transcript decreased, and, significantly, the Kv1.4 transcript remained constant compared with ventricle (Table 2).

The value of the total Kv1 subfamily message level was also compared between atria and ventricle (Table 2). In this case, the total Kv1 level in ventricle was normalized to equal 100. The total amount of Kv1 subfamily mRNA was \( \approx 73\% \) higher in atria than in ventricle (Table 2).

**Kv4 Gene Family**

For the purposes of comparison, the amount of Kv4.2 transcript in ventricle was arbitrarily given a value of 100, and the abundance of the Kv4.1 mRNA was expressed relative to this value (Table 2). The Kv4.2 transcript was by far the predominant Kv4 subfamily mRNA transcript in both tissues. The contribution of the Kv4.1 gene to the total Kv4 mRNA was small or
Within also isolated and used was RNA (Fig 4). The relative level of expression of the Kv1 and Kv4 gene families was also determined. The ratio of Kv4.2 to Kv1.2 expression in ventricle was 1.6±0.5. This result suggests that the relative abundance of the Kv1 and Kv4 family transcripts is roughly comparable.

**Heterogeneity of Potassium Channel Expression Within the Ventricular Wall**

There is clear evidence from electrophysiological studies that there is a gradient of expression of a rapidly inactivating potassium conductance (known as \( I_\text{to} \)) across the ventricular wall, with expression being highest in the epicardium and lowest in the endocardium and papillary muscle.\(^{16,17} \) To test whether these differences could be due to differences in mRNA expression, we tested RNA samples obtained from four layers of the left ventricular wall: (1) papillary muscle, (2) the endocardial region, (3) the midregion of the ventricular wall, and (4) the epicardium. The right ventricular wall was also isolated and used for RNA preparation.

The Kv4.2 mRNA was found to be expressed in a steep gradient across the left ventricular wall (Fig 4A), with expression being 8.4 times higher in epicardium than in papillary muscle (Fig 4D). This difference in Kv4.2 expression was the largest difference for any comparison of potassium channel expression in cardiac muscle.

In contrast to the results for Kv4.2, there were relatively minor differences in the abundance of the Kv1.2, Kv1.4, Kv1.5, and Kv2.1 transcripts across the ventricular wall (Fig 4). There were small decreases in the expression of the Kv1.2 and Kv1.5 genes and small increases in the expression of the Kv1.4 and Kv2.1 genes. The pattern of expression of potassium channel transcripts in the right ventricle was most similar to the expression in the left ventricular epicardium (Fig 4D). Because the gradient of Kv4.2 expression across the ventricular wall could potentially be compensated for by a reverse gradient of Kv4.1 transcripts, Kv4.1 mRNA levels were also tested. The Kv4.1 transcripts were found to be uniformly very low in all four tissue samples.

In another set of experiments, the left ventricular wall was split into thirds in a latitudinal direction, and samples were prepared from the base third, central third, and apical third. In this experiment, no large differences in potassium channel expression between the base and apex of the ventricular wall were seen (data not shown).

**Expression of IsK**

Although this channel does not have the typical six-membrane-spanning domain architecture of the other voltage-gated potassium channels, it does appear to produce a voltage-gated potassium channel in heterologous expression systems\(^{38,50} \) and we have included it in the analysis for the sake of completion. A very weak, but specific, IsK hybridization signal was found in both atrial and ventricular RNA samples after long exposures. There was no obvious difference in the expression of this gene between the two tissues (data not shown). Transcripts from the IsK gene are so rare that it is relatively unlikely that the products of this gene make a significant contribution to the potassium channels expressed in adult rat cardiac myocytes. The IsK gene transcript has previously been reported not to be present in adult rat heart.\(^{38} \)

**Contamination of Heart Samples by Parasympathetic Neurons**

The possibility that the atrial or ventricular RNA samples were contaminated with RNA from parasympathetic neurons was addressed in the following way. RNA was isolated from three different tissue samples: (1) atrial appendage (the normal atrial dissection used in the present study), (2) atrial septum (which should contain the bulk of parasympathetic neurons), and (3) bottom of the ventricle (the normal ventricular dissection). We tested for the presence of neuronal RNA by testing for the expression of the \( \beta_4 \) subunit of the neuronal acetylcholine receptor, which is a major subunit of acetylcholine receptors in peripheral neurons.\(^{51} \) For the heart samples, a specific \( \beta_4 \) signal could be detected only in the atrial septum sample (Fig 5), as expected from anatomic studies on the distribution of the cardiac gan-

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**Fig 3.** Comparison of Kv3 potassium channel expression in atrial and ventricular muscle by RNase protection analysis. A shows subunits Kv3.2 and Kv3.4; B, subunit Kv3.1; and C, subunit Kv3.3. In these experiments, the cardiac muscle samples contained 10 \( \mu \)g of total RNA, and the positive control brain sample contained only 2.5 \( \mu \)g of total RNA. A specific signal could always be seen in the positive control samples after an overnight exposure to x-ray film. Vent indicates ventricle.
Glia. This suggests that the cardiac muscle samples used in the present study are essentially free of contaminating parasympathetic neurons. It should be noted that even in the atrial septum sample, the β signal was very weak, which is consistent with the relatively small number of neurons in the cardiac ganglia.

Discussion

In the present study, the expression of 15 potassium channel genes in rat atrial and ventricular muscle was determined by an RNase protection assay. In the following discussion, we will attempt to correlate the mRNA expression results with previous electrophysiological results. It is fully appreciated that there is not necessarily a simple linear relation between mRNA expression levels and the number of functional channels expressed in the cell membrane. Similarly, the effect of heteromultimer formation will complicate the interpretation of the results.

Voltage-clamp studies have shown that the basic difference in the properties of voltage-gated potassium channels expressed in rat atrial and ventricular myocytes is that the total outward current inactivates more rapidly in ventricular myocytes than in atrial myocytes. The results from the mRNA analysis are in general agreement with this observation. The potassium channel mRNA species that change the most in relative abundance in comparisons of atrial and ventricular RNA samples are transcripts from the Kv1.2 and Kv4.2 genes. The most unambiguous difference is the twofold higher level of expression of the Kv4.2 gene in ventricular muscle compared with atria. The protein products of the Kv4.2 gene form a rapidly inactivating channel, and the higher level of expression of Kv4.2 in ventricle is consistent with the relative abundance of rapidly inactivating potassium channels in isolated ventricular myocytes.

The next largest difference in comparisons of atria and ventricle is in the expression of the Kv1.2 gene. This change is more difficult to interpret, however, because of uncertainties about the kinetic properties of the Kv1-class channels found in cardiac muscle. The level of expression of the Kv1.2 gene, which encodes subunits...
that form a very slowly inactivating channel, is increased more than twofold in atria compared with ventricle, and there is a relative increase in the level of Kv1.2 transcripts over the Kv1.4 and Kv1.5 transcripts (Table 2). There is, however, a relative decline in the number of Kv1.5 transcripts (Table 2), the products of which also form a slowly inactivating channel. These changes will tend to counteract each other in terms of their possible effects on the properties of Kv1 heteromultimers. Most important, there is little or no change in the relative level of expression of the Kv1.4 gene, which encodes a rapidly inactivating potassium channel subunit. Overall, there is a net increase in the abundance of Kv1 subfamily transcripts in atria compared with ventricle because of increases in the transcription of all three Kv1 genes (Table 2). Because of the possibility of heteromeric formation, the predominant kinetic characteristics of the Kv1 channels remain difficult to predict.

Within the left ventricular wall, there is a large gradient of Kv4.2 expression, whereas the level of expression of the other transcripts does not vary greatly (Fig 4). There is an evident parallel between this observation and the gradient of \( I_{Na} \) expression that has previously been found in cats, dogs, and rabbits. There are no directly comparable electrophysiological studies of rat ventricular myocytes isolated from different regions of the left ventricular wall. It is well established, however, that there is significant variability in the amount of \( I_{Na} \) expressed by rat ventricular myocytes isolated in bulk from the ventricular wall, suggesting that there may be regional differences in potassium channel expression in rat ventricle similar to those seen in other species.

There is one clear discrepancy in the results from studies of potassium channel mRNA expression compared with previous studies of rat myocytes in which voltage-clamp techniques were used. The results from the present study predict that there should be at least three distinct voltage-activated potassium currents expressed in atrial and ventricular myocytes, corresponding to the products of the Kv1, Kv2, and Kv4 gene families. Three distinct voltage-gated potassium currents have not been found in rat atrial and ventricular myocytes. It is notable in this context, however, that single-channel recording techniques can distinguish more voltage-gated potassium channels in ventricular myocytes than can be separated by whole-cell voltage-clamp analysis.

Potassium channel blockers, which block only a fraction of the total potassium current in cardiac myocytes, can produce significant changes in the shape of the cardiac action potential. The sensitivity of the action potential to changes in the number of functional potassium channels suggests that maintenance of stably differentiated action potentials in atrial and ventricular myocytes requires tight regulation of the expression of potassium channel genes in these cells. The results in the present study are consistent with this suggestion. There did not appear to be any example of a simple on or off regulation of any of the potassium channel genes expressed in atrial and ventricular muscle, and the magnitude of the differences in mRNA levels were concordant with the differences in potassium currents seen when voltage-clamp techniques were used. Although there may be other mechanisms that regulate potassium channel expression in cardiac muscle, it would appear that transcriptional regulation is an important component of this process.

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