Spatial distributions of Kv4 channels and KChip2 isoforms in the murine heart based on laser capture microdissection

Christine Teutsch a,c,1, Richard P. Kondo b,c, Dorothy A. Dederko a, Jacqueline Chrast c,2, Kenneth R. Chien c,1, Wayne R. Giles a,c,*

a Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA
b Department of Medicine, University of California, San Diego, La Jolla, CA, USA
c Institute of Molecular Medicine, University of California, San Diego, La Jolla, CA, USA

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Abstract

Objective: Regional differences in repolarizing K⁺ current densities and expression levels of their molecular components are important for coordinating the pattern of electrical excitation and repolarization of the heart. The small size of hearts from mice may obscure these interventricular and/or transmural expression differences of K⁺ channels. We have examined this possibility in adult mouse ventricle using a technology that provides very high spatial resolution of tissue collection.

Methods: Conventional manual dissection and laser capture microdissection (LCM) were utilized to dissect tissue from distinct ventricular regions. RNA was isolated from epicardial, mid-myocardial and endocardial layers of both the right and left ventricles. Real-time RT-PCR was used to quantify the transcript expression in these different regions.

Results: LCM revealed significant interventricular and transmural gradients for both Kv4.2 and the alpha-subunit of KChIP2. The expression profile of a second K⁺ channel transcript, Kir2.1, which is responsible for the inwardly rectifying K⁺ current Iₖᵢ, showed no interventricular or transmural gradients and therefore served as a negative control.

Conclusions: Our findings are in contrast to previous reports of a relatively uniform left ventricular transmural pattern of expression of Kv4.2, Kv4.3 and KChIP2 in adult mouse heart, which appear to be different than that in larger mammals. Specifically, our results demonstrate significant epicardial to endocardial differences in the patterns of expression of both Kv4.2 and KChIP2.

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1. Introduction

The adult mammalian heart exhibits regional heterogeneities of ventricular action potential (AP) waveforms. These differences modulate both the propagation of cardiac excitation and the pattern of repolarization. An important axis of this electrophysiological heterogeneity is the transmural gradient of action potential duration across the free wall of both the right ventricle (RV) and the left ventricle (LV) [1]. The AP of the LV epicardium has a shorter duration and a more prominent early repolarization phase compared to the AP of the LV endocardium [2,3]. The second important axis of heterogeneity is the difference in action potential duration between the RV and LV [4]. The action potential duration of the RV epicardium is shorter than the LV epicardium and the notch in early repolarization of the AP is deeper and more...
prominent. Thirdly, there is the apex–base axis of action potential duration [5].

The principle mechanism by which this diversity of AP waveforms and durations is created is through regional heterogeneities of K+ currents (for overview see [6]). \(I_{\text{to}}\), a Ca\(^{2+}\)-independent transient outward K\(^+\) current, which is responsible for early repolarization, has been well-documented to be expressed in a heterogeneous manner throughout the ventricles of mammalian hearts. This pattern is concordant with known AP duration differences [7–9].

\(I_{\text{to}}\) heterogeneity and its underlying molecular basis have been discussed extensively in the literature. Furthermore, substantial differences among the mammalian species have also been reported [10]. \(I_{\text{to}}\) can be divided into \(I_{\text{to(fast)}}\) and \(I_{\text{to(slow)}}\). Kv4 and KChIP2 are the molecular basis for the \(I_{\text{to(fast)}}\) current, while Kv1.4 mediates \(I_{\text{to(slow)}}\) [11–13]. In human and ferret both \(I_{\text{to(fast)}}\) and \(I_{\text{to(slow)}}\) are expressed in the transmural aspect of the ventricular walls: \(I_{\text{to(fast)}}\) expression is highest in the LV epicardium, whereas that of \(I_{\text{to(slow)}}\) is highest in the LV endocardium. In canine only \(I_{\text{to(fast)}}\) appears to be present in the LV wall (for overview see [10]). Although there is heterogeneous expression of both currents (\(I_{\text{to(fast)}}\) and \(I_{\text{to(slow)}}\)) in the mouse heart, \(I_{\text{to(slow)}}\) is present only in the interventricular septum. In contrast, expression of \(I_{\text{to(fast)}}\) has been identified in the apical–basal, epicardial–endocardial and interventricular axes of the adult murine heart [9,11,12]. The focus of this study of the murine heart is on \(I_{\text{to}}\), which is analogous to the \(I_{\text{to(fast)}}\) current in other species.

Variations in K+ current densities may reflect transcriptional differences, alterations in protein expression, and/or variations in the functional properties of the K+ channels. The molecular correlates of the pore-forming \(\alpha\)-subunits of \(I_{\text{to}}\) have been identified as Kv4.3 in large mammals and Kv4.3/Kv4.2 in rodents [13–16]. In addition, Kv4 channel expression and function are modulated by regulatory subunits (\(\beta\)-subunits). The Kv Channel Interacting Proteins (KChIPs) are a family of such \(I_{\text{to}}\) modulating \(\beta\)-subunits. KChIP2, the primary isoform in the mammalian ventricle, has been shown to increase the amplitude of this current and alter its gating kinetics [17–20]. The importance of KChIPs in \(I_{\text{to}}\) expression has been illustrated by the absence of \(I_{\text{to}}\) in mice lacking the KChIP2 gene [21].

The present consensus in the literature is that \(I_{\text{to}}\) current density gradients in human and canine are due to gradients in KChIP2 expression. In contrast, findings from rats and mice have been interpreted in terms of the \(I_{\text{to}}\) gradient being due to heterogeneous expression of Kv4.2, but not KChIP2 [22–24].

We sought to examine the spatial expression gradients of the gene products responsible for the \(I_{\text{to}}\) current in the adult mouse heart. The small size of the mouse heart presents limitations in the ability to assess expression variations across the very thin ventricular wall. Accordingly we proposed that the spatial resolution of the method used for tissue collection may impact the outcome of studies of the murine heart. In this study we obtained region-specific murine heart tissue using two methods: (1) standard manual dissection and (2) laser capture microdissection (LCM), which yields very thin myocardial sections (80–100 \(\mu\)m width). Quantitative RT-PCR was utilized to measure the relative expression profiles of Kv4.2, Kv4.3 and KChIP2. As a negative control the inwardly rectifying K+ channel, Kir2.1 was studied. It did not show transmural variations.

2. Methods

Male C57B6 mice, 8–10 weeks of aged, were used in all experiments. All aspects of this investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication NO. 85-23, revised 1996). Adult mice were sacrificed using a sodium pentobarbital overdose. The hearts were rapidly excised and excess blood removed by blotting with Kimwipe paper. The heart dissection protocol was approved by the Animal Subjects Committee at the University of California, San Diego.

2.1. Manual dissection of tissue strips from different locations of the right and left ventricle

Following excision of the heart, both the RV and LV free walls were rapidly removed and cut into apical, mid and basal portions. The middle regions of these strips were discarded. The apical and basal regions from the LV free wall were further divided into epicardial and endocardial layers as follows. The LV apical and basal tissue strips were pinned to a polymer-based surface with the epicardial surface facing upwards. A thin epicardial layer, approximately 1.5–2 mm in thickness (visual inspection), was dissected and retained. The underlying mid-myocardial layer was removed and discarded. The remaining endocardial layer was retained. All tissue strips were rapidly frozen in liquid nitrogen. For each set of RNA isolation the dissected tissue strips from 3 hearts were pooled.

2.2. Laser capture microdissection (LCM)

Immediately following excision, the heart was perfused via the aorta in a retrograde fashion with 5 ml PBS solution (phosphate-buffered saline solution), containing 1 U/\(\mu\)l RNAsin (Promega). This potent RNAse-inhibitor protected the RNA from degradation. Subsequently, the heart was embedded in Tissue-Tek O.C.T. Compound (WR International, Bridgeport, NJ) and frozen in liquid nitrogen. Following embedding, the frozen heart was sectioned along the coronal plane in 16-\(\mu\)m-thick cryosections using a Jung Frigocut 2800E cryostat (Leica). These cryosections were placed on slides specifically designed for the laser capture apparatus (Molecular Machines and Industries). The integrity of RNA extracted from laser microdissected sections is dependent upon the length of time that the cryosections are exposed to room temperature [25]. To minimize the exposure of the cryosections to ambient air, only one
The cryosection was fixed per slide and the slide was then rapidly placed on dry ice. The cryosections were fixed on the slides in 70% ice-cold ethanol for 1 min, stained with hematoxylin and eosin and then gradually dehydrated using increasing concentrations of ethanol. Dehydration was completed by incubation in xylene for 10 min and air drying for at least 5 min in a hood. All solutions were made with DEPC-treated water and RNAs in (Promega) was added (1 U/μl) to prevent any RNA degradation. Laser microdissection was performed with the μCUT Laser Microdissection System (Molecular Machines and Industries AG; wavelength: 355 nm), which permits the calibrated measurement and determination of the dimensions of the dissected areas. The maximum thickness of the tissue strips was set between 80 and 100 μm (Fig. 1), which correlates to about 3–4 myocyte layers (Fig. 1E). In order to sample equal amounts of tissue from each region (RV epicardium, RV endocardium, LV epicardium, LV mid-myocardium and LV endocardium), the total area that was captured per sample was measured with the μCUT Laser Microdissection System Software, and always kept in a range of 700,000–800,000 μm². This corresponds to the epicardial and endocardial layers from apex to base of both ventricles of one 16-μm cryosection. The tissue strips were rapidly separated from the surrounding tissue using tubes with adhesive lids (Molecular Machines and Industries) and immediately immersed in lysis buffer (Qiagen RNA-MicroIsolation Kit).

2.3. RNA isolation and quantitative RT-PCR analysis

Quantitative RT-PCR was performed to determine the relative mRNA expression patterns of Kv4.2, Kv4.3, KChIP2 and Kir2.1 in the RV, LV epicardium and LV endocardium. Total RNA from manually dissected tissue strips was isolated using the Trizol™-reagent (Gibco-BRL) [26]. Any contaminating genomic DNA was removed by on-column DNase digestion (Qiagen). Total RNA from tissue strips generated by LCM, was isolated using the RNA MicroIsolation Kit (Qiagen), which also includes an on-column DNA digestion step. First-strand cDNA synthesis and real-time PCR amplification were done with the Platinum® qRT-PCR ThermoScript™ One-Step System (Invitrogen) and the TaqMan® Assays-on-Demand™ primer/probes pairs specific for GAPDH, Kv4.2, Kv4.3, KChIP2 and Kir2.1 (Mm99999915_g1, Mm00498065_m1, Mm00498260_m1, Mm00518914_m1, Mm00434616_m1 Applied Biosystems) [27,28]. Optimal PCR curves were observed within 40 cycles using the Mx4000® Multiplex Quantitative PCR System (Stratagene). The threshold value (Ct) was set with the amplification-based threshold algorithms from the Stratagene Mx4000® Multiplex System Software. GAPDH expression of

Fig. 1. Histological images demonstrating laser capture microdissection (LCM) of tissue strips from specific regions of the right and left ventricles of adult mouse hearts (A–E). Cryosections (16-μm thickness) prepared for LCM (see Methods), with eosin–hematoxylin staining to visualize myocyte cytoplasm and nuclei. (A and C). Laser microdissection of epicardial tissue slices from the surrounding tissue of the right (panel A) and left (panel C) ventricular epi- and sub-epicardium (width: 80–100 μm as calculated by the μCUT Laser Microdissection System). (B and D). Isolated right (panel B) and left (panel D) epi- and sub-epicardial tissue slices that were collected for RNA isolation. (E) LCM tissue strips at 20× magnification show myocytes and nuclei. The 80- to 100-μm-thick tissue strips consist of a single layer of epicardium with 2–3 additional sub-epicardial layers of myocytes. LCM tissue strips were obtained from endo- and sub-endocardial regions of the right and left ventricle (data not shown).
each sample was used as an endogenous control. For the manually dissected tissue slices, the height of histogram columns represent the fold-change of transcript expression in 80 ng of the isolated RNA from the selected locations, relative to the transcript expression in 80 ng whole heart RNA (reference sample or calibrator). Means of the data from three separate manual RNA isolations are shown. Three hearts were pooled for each isolation. Quantitative RT-PCR measurements for the manually dissected tissue slices were also performed in triplicate. For the LCM tissue strips, the histogram columns represent the fold-change of transcript expression of the RNA content of 200,000 μm² tissue from the selected locations relative to the transcript expression of 200,000 μm² tissue whole heart RNA (calibrator). In this case, three separate RNA isolations were included in the histogram. That is, each isolation represents one animal. For each isolation 3 sets of laser captured tissue from 16-μm-thick cryosections were pooled. The quantitative RT-PCR measurements were done in duplicate. Negative controls for contamination artefacts were performed, using Taq-Polymerase (Invitrogen) with the TaqMan® primers/probes pairs, but without the reverse transcription step. These steps revealed no product amplification.

2.4. Efficiency of amplification

The efficiency of target genes and GAPDH amplification was evaluated using standard curves derived from on serial dilutions of RNA. For the manually dissected tissue slices, amplification curves for GAPDH and the target genes were generated using 320, 80 and 20 ng of total whole heart RNA. For the LCM tissue strips 50–500 pg RNA was used to generate standard amplification curves. Three sets of RNA isolations from each dissection method were evaluated and each measurement was performed in triplicate. The target genes as well as the GAPDH TaqMan® Assays-on-Demand™ primer/probes pairs had comparable amplification efficiencies (range between 85% and 110%).

2.5. Quality assessment of manually and LCM extracted RNA

A series of control experiments was done to assess differences in RNA quality between the two dissection techniques, since the extensive processing of the laser microdissected slices might effect RNA quality. Two sets of RNA were isolated, one directly after manual dissection of the heart (MA-RNA), and the other after cryosectioning and staining protocols (LC-RNA). This represented the LCM treatment. MA-RNA: murine whole heart tissue was manually dissected and RNA isolated as described above. LC-RNA: murine whole heart was processed as described above for LCM. Since the amount of RNA extracted from laser microdissected tissue strips is not sufficient for these quality assessments, tissue from about 20 stained and dehydrated sections was pooled and RNA was isolated using the RNA Mini-Isolation Kit with on-column DNAse digestion (Qiagen). These 20 pooled cryosections were processed and dehydrated exactly as described in the LCM protocol. We therefore considered further RNA degradation relatively unlikely to occur during the final steps of LCM, i.e., during the laser microdissection of the tissue on the µCUT Laser Microdissection System. In order to compare the RNA quality of MA-RNA and LC-RNA, the MA-RNA was diluted to match the RNA concentration obtained by pooling of the 20 cryosections (LC-RNA). A NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies) was used to measure UV absorbance spectra and 260/280 absorbance ratios and concentrations (Fig. 4A). To further assess the RNA quality, quantitative RT-PCR amplification curves of serial RNA dilutions of the MA-RNA and LC-RNA were run and the reaction efficiencies (value for RNA quality) for the different target genes were compared.

2.6. Data analysis

All data are presented as means ± standard deviations. Expression levels were analyzed for statistically significant differences between the selected regions (all regions together, RV and LV) using one-way ANOVA (Origin 6.1, OriginLab Corp., Northampton MA). If ANOVA analysis indicated an overall difference between group means, Bonferroni post hoc tests were performed to determine whether group means differed significantly.

3. Results

3.1. mRNA expression profiles for Kv4.2 and Kv4.3 in mouse ventricle

In order to assess the transmural, interventricular and apex—base distributions of K⁺ currents, mRNA transcripts levels of Kv4.2 and Kv4.3 α-subunits were obtained using quantitative RT-PCR. Hearts from adult male mice were dissected to yield six consistent sets of slices: apical and basal regions of the right ventricular wall, as well as epicardial and endocardial layers of the apical and basal regions of the LV. Fig. 2A shows the relative expression levels of Kv4.2. Note that the mRNA transcript levels for Kv4.2 are significantly higher in the RV than in any region of the LV. Kv4.2 expression is also greater in the epicardium compared to the endocardium of the LV. In contrast, there was no difference in the Kv4.2 expression level between comparable apical and basal regions. A similar approach was used in an attempt to identify the patterns of Kv4.3 expression. There appeared to be a small reduction (15%) in the Kv4.3 transcript level in the apical region of the LV compared to the other regions (Fig. 2B). However, in most region no statistically significant patterns were identified.

3.2. mRNA expression profile for Kir2.1 in mouse ventricle

The spatial distribution of Kir2.1 expression in manually dissected tissue was assessed to determine the expression pattern of the molecular correlate of the inward rectifier K⁺
Previous papers have reported little or no regional variation in adult mouse heart for this transcript [24,29,30]. Our findings confirm this. In contrast to Kv4.2, there was no statistically significant difference in Kir2.1 expression between any of the selected regions in adult mouse ventricles (Fig. 2C).

3.3. mRNA expression profile of KChIP2 in mouse ventricle

KChIP2 can act as a chaperone for some Kv4 ion channels. This transcript can also alter the kinetics of inactivation and recovery from inactivation of Kv4 channels [17–20]. Targeted deletion of the KChIP2 gene in mouse causes loss of the Kv4.2 mediated K⁺ current, I_K. This suggests that KChIP2 is essential for functional expression of Kv4.2/ Kv4.3 in mammalian hearts [21].

Multiple splice variants for KChIP2 (a–f) have been identified [18,20,21]. The predominant KChIP2 isoforms expressed in mouse ventricle are KChIP2a and KChIP2b [21]. We used quantitative RT-PCR primers, which amplify both of these isoforms to obtain the pattern of expression of KChIP2a/2b. Our results revealed a small, but statistically significant reduction of KChIP2a/2b in LV endocardium compared to LV epicardium (Fig. 2D). There were also small, statistically significant differences between sub-regions of the RV compared to sub-regions of the LV (RV base versus RV apical region only).
LV base/epi). However, no apex–base differences of KChIP2a/2b expression were identified.

3.4. Spatial profile of expression of Kv4.2 and KChIP2 based on laser capture microdissection

Expression profiles based on RNA derived from manually dissected epicardial and endocardial ventricular tissue slices can be influenced by the limited spatial resolution of the manual dissection procedure. This limitation is important to consider in the analysis of spatial gene expression heterogeneity in small tissues, such as the mouse heart. The maximal transmural thickness of the adult mouse left ventricle is approximately 2.5 mm. Each manually dissected tissue slice has a thickness of about 1.0–1.5 mm. To determine whether our previous results were compromised by the limited spatial resolution of manual dissection, laser capture microdissection (LCM) was used to obtain 80- to 100-μm-thick tissue strips from the right and left ventricular free walls (Fig. 1). As shown in Fig. 1E, these tissue strips consist of only the epicardial cell layer with 2–3 additional sub-endo/epicardial cell layers. This significant improvement in spatial resolution is especially valuable in the analysis of transmural expression patterns of the murine RV. Reproducible separation of right ventricular epicardial and endocardial regions is not possible with conventional manual dissection methods.

A detailed comparison of epicardial and endocardial slices microdissected from the RV wall revealed a RV transmural gradient of Kv4.2 (Fig. 3A). In addition, a gradually decreasing expression profile of Kv4.2 from epicardium to endocardium across the LV free wall was consistently observed (Fig. 3A). Note that the Kv4.2 expression gradient in the RV transmural orientation was consistently steeper when using microdissected layers rather than the manually dissected slices. Specifically, the LCM results showed a 5.8-fold larger expression of Kv4.2 in the LV epicardium compared to the LV endocardium. Only a 2-fold difference was seen when using manually dissected slices.

When similar microdissected slices were used to assess the expression profile of KChIP2, a significant transmural gradient of this transcript was also detected in both RV and LV (Fig. 3B). There was a 1.6-fold higher KChIP2 expression in the LV epicardial slices compared to the LV endocardial

Fig. 3. Expression profiles for the murine Kv4 channel α-subunits and β-subunit obtained from laser capture microdissected sections. Quantitative RT-PCR was performed to determine the relative mRNA expression patterns of Kv4.2, KChIP2 and Kir2.1 in the RV epicardium and endocardium and in the LV epicardial and LV endocardial. The average thickness of microdissected tissue strips was between 80 and 100 μm. All histogram data are presented as mean±standard deviation. In these histograms the vertical axes represent the fold-change of transcript expression of the RNA content of 200,000 μm² tissue from the selected locations relative to the transcript expression of 200,000 μm² tissue whole heart RNA (reference sample or calibrator). (A) Expression profiles of Kv4.2. The Kv4.2 expression is significantly larger in the RV epicardium compared to RV endocardium. Kv4.2 expression is significantly larger in LV epicardium compared to LV mid-myocardium, while LV mid-myocardial expression of Kv4.2 is significantly larger than in LV endocardium (N=6, mean±SD; one-way ANOVA, **p<0.01). Kv4.2 expression is also significantly higher in the RV epicardium compared to RV endocardium. (B) Expression profiles of KChIP2. KChIP2 expression is significantly higher in the RV epicardium compared to RV endocardium. In addition, KChIP2 expression is significantly larger in LV epicardium compared to LV mid-myocardium and LV mid-myocardial expression of KChIP2 is significantly greater than in LV endocardium (N=6, mean±SD; one-way ANOVA: *p<0.01). (C) Expression profiles of Kir2.1. There were no significant differences of expression of Kir2.1 (N=6, mean±SD; one-way ANOVA).
slices. Similarly, there was a 1.4-fold higher KChIP2 expression in the RV epicardial slices compared to the RV endocardial slices. In contrast, Kir2.1 expression did not exhibit any differences either in the interventricular or transmural aspects (Fig. 3C), based on data from the same LCM.

An analogous study of the Kv4.3 expression levels was attempted. However, no amplification was observed, suggesting that the transcript level in the laser microdissected sections was insufficient to be detected reliably.

3.5. Assessment of effects of tissue preparation for LCM on mRNA quality

The extensive tissue processing required for the LCM method could, in principle, influence subsequent RNA measurements, and compromise meaningful comparisons of the two dissections methods. Thus, control experiments were done to address this possibility. Two sets of RNA were isolated, one directly after manual dissection of the heart (referred to as: MA-RNA), and the other after completing cryosectioning and staining protocols (referred to as: LC-RNA). Fig. 4B shows that the RNA quality of both data sets (MA-RNA and LC-RNA) is comparable based on UV absorbance spectra, with slightly larger absorbance ratios (at the wavelengths 260/280) for the MA-RNA (1.72) compared to LC-RNA (1.61). To date there is no simple metric to predict whether the mRNA is intact, especially in very small samples. Although total RNA with a 28S:18S rRNA ratio between 1.5 and 2.0 denotes high quality, the relationship between rRNA profile and mRNA integrity is still unclear. Since we were specifically interested in the condition and the quality of the mRNA in our samples, we utilized real-time RT-PCR for this approach. Serial RNA dilutions of both the MA-RNA and LC-RNA isolations were amplified with real-time RT-PCR, to compare the amplification profiles of these transcripts, and on contradicting data presented in the literature [32,23].

There is now a general consensus that in human and canine ventricles, $I_{\infty}$ current gradients are regulated by KChIP2 expression differences [22,23]. Human and canine KChIP2 expression gradients have been demonstrated in all three axes, epi-endo, apex–base and RV-LV. However, although Kv4.3 was originally reported to be expressed uniformly throughout mammalian ventricular tissue [23], Zicha et al. identified transmural Kv4.3 mRNA and protein expression gradients in the canine LV [33]. This paper also raised questions about the importance of KChIP2 for establishing/maintaining $I_{\infty}$ gradients in the canine heart. In the ferret heart, all three transcripts, Kv4.3, Kv4.2 and KChIP2, are expressed in a heterogeneous manner throughout the ventricular walls. Significant Kv4.2 and KChIP2 gradients have been described in the epi to endo and right to left axes. This distribution suggests that both genes, Kv4.2 and KChIP2, are important for $I_{\infty}$ current density gradients in ferret hearts [8,34].

In the adult mouse heart a consensus has emerged, that $I_{\infty}$ gradients are due to heterogeneous expression of Kv4.2 (LV epi $\rightarrow$ LV endo) but that no such gradients are present with respect to KChIP2 expression [35]. However, no heterogeneity of Kv4.2 expression has been shown along the apex–base axis of the heart or between the ventricles, emphasizing that Kv4.2 expression patterns cannot explain all $I_{\infty}$ gradients.

Our results demonstrate that there are transmural (RV and LV) and interventricular gradients of Kv4.2 expression in the adult mouse heart. KChIP2 also shows transmural gradients in both the RV and the LV. However, neither Kv4.2 nor KChIP2 exhibits an apex–base gradient of expression. We also failed to detect spatial gradients in the other selected $K^+$ channel genes. These results support the hypothesis that Kv4.2 and KChIP2 expression differences contribute to $I_{\infty}$ gradients along two functional axes but not the third (apex–base), in the ventricles of the adult mouse heart.

4.2. Comparison to known regional heterogeneity of electrophysiological properties

It is well documented in mammalian hearts that there is marked $I_{\infty}$ current density gradient between the LV epicardium and the LV endocardium. Such transmural density gradients have been reported for rat, rabbit, dog, ferret and human (for overview see [10]). Additionally, there is evidence for two other axes of $I_{\infty}$ current expression: (i) apex to base, with higher densities in apical myocytes, and (ii) interventricular, with higher densities in the RV versus the LV [2–5,31].

At a molecular level, Kv4.2, Kv4.3 and KChIP2 are generally accepted as molecular correlates of the $I_{\infty}$ current. However, the importance of each transcript for the current density gradients along each axis continues to be debated and discussed. These discussions are mainly based on species-specific expression differences in RNA versus protein levels of these transcripts, and on contradicting data presented in the literature [32,23].

4.1. Summary of main findings

Our results demonstrate that there are transmural (RV and LV) and interventricular gradients of Kv4.2 expression in the adult mouse heart. KChIP2 also shows transmural gradients in both the RV and the LV. However, neither Kv4.2 nor KChIP2 exhibits an apex–base gradient of expression. We also failed to detect spatial gradients in the other selected $K^+$ channel genes. These results support the hypothesis that Kv4.2 and KChIP2 expression differences contribute to $I_{\infty}$ gradients along two functional axes but not the third (apex–base), in the ventricles of the adult mouse heart.
Fig. 4. Assessment of quality of the RNA isolated from manual dissected tissue samples and from tissue samples prepared using LCM. Two sets of RNA were isolated, one directly after manual dissection of the heart (MA-RNA), the other after cryosectioning and staining protocols (LC-RNA), representing the treatment performed for LCM. (Panel B) Comparison of absorbance spectra of MA-RNA and LC-RNA (NanoDrop® ND-1000 Spectro-photometer, NanoDrop Technologies). Note that these absorbance curves were very similar at the chosen RNA concentration. The absorbance ratios at the wavelengths 260/280 are slightly higher for the MA-RNA (1.72) compared to LC-RNA (1.51). (Panel A) Comparison of quantitative RT-PCR amplification curves of GAPDH in serial RNA-dilutions from MA-RNA and LC-RNA. (C) Comparison of the reaction efficiency for GAPDH amplification as determined from the amplification curves of the serially diluted RNA. The slopes of these lines are very similar. This indicates that the reaction efficiency with both RNA isolation is nearly 100%.
significant apex–base differences for the K⁺ channel α-subunits could be detected. Additionally our observation of a right ventricular transmural Kv4.2 gradient in the LCM based expression analysis suggests corresponding electrophysiological differences. The thin wall of the RV poses a significant challenge to convincingly separate RV epicardial and endocardial myocytes for electrophysiological studies and transcript analysis. However, both human and dog exhibit RV transmural gradients of the transient outward K⁺ current [23,36].

Our observation of a significant transmural gradient of KChIP2 in the LV of the adult mouse heart is an important finding in attempts to understand the causes and significance of transmural Iₒ density gradients. Our results disagree with previous studies, which reported uniform KChIP2 expression in the ventricles of adult mouse hearts. However, it should be noted that previous studies, which failed to detect a KChIP2 mRNA transmural gradient in mouse LV, relied on manually dissected slices. These results are therefore comparable to our findings shown in Fig. 3. The KChIP2 gradient appears to be less pronounced than the Kv4.2 gradient. In fact, the KChIP2 gradient could only be detected with the higher spatial resolution offered by LCM.

The small but consistent (1.6-fold) gradient for KChIP2, which we have observed in the murine ventricle is comparable to the gradients for Iₒ measured using voltage clamp methods. Specifically, in the adult mouse heart Iₒ expression is approximately 1.7 times larger in the left ventricular epicardium compared to the endocardium [9]. We note that this ratio differs significantly from the values reported from studies in larger mammals where Iₒ peak current expression gradients vary from 3.4–4.1 in human, to 7.5–10 in canine ventricles. The reasons for these differences are not known. However, species differences may contribute. Technical factors associated with tissue collection (and hence spatial resolution) also need to be considered when attempting to compare molecular or electrophysiological data from mouse, dog and human.

Previous studies have focussed on these expression gradients for their functional importance, i.e., their ability to regulate the target current, Iₒ. A well-defined linear correlation between KCHIP2 mRNA and Iₒ current densities has been reported in the canine LV (r² = 0.94) [23]. This result is consistent with the finding that the Kv4.2/4.3 alpha subunit and KChIP2 complex exhibit a 1:1 stoichiometry, with 4 subunits of Kv4.3 binding to 4 KChIP2 molecules to form a functional channel. However, it also has been reported that the presence of KCHIP2 subunits can increase the amplitude of Kv4 currents 10- to 30-fold [17]. Thus, a modest increase in KCHIP2 expression can have a significant effect on the size of cardiac Kv4 currents. A correlation between known subunits is therefore unlikely to represent a unique cause and effect relationship. Furthermore, it is important to note, that in addition to the KCHIP2 gene product, Kv4.3 also exhibits a transmural expression gradient in canine and human ventricles [33]. This, together with the fact that KCHIP2 functions as a chaperon for the transportation of Kv4.2 to the sarcolemma of the myocyte, emphasizes that the observed Iₒ gradients can result from a number of interacting mechanisms. Indeed, the expression gradients of the molecular components represent only one component of a multifactorial regulation mechanism.

4.3. Limitations

A limitation of this study is that it assesses only differential expression of mRNA levels as opposed to protein values. Nevertheless, real-time RT-PCR is the best technology available for quantification of putative expression differences in such small tissue samples. In previous studies, which have compared levels of mRNA with levels of protein expression, a close correlation between transcript signal and size of the encoded current has been reported [23,32].

LCM is a technique used to isolate and dissect single cells, on which RT-PCR can be performed. Unfortunately, only relative quantification is possible with this approach. Using LCM to capture sufficient tissue to generate the required quantities of RNA needed for real-time RT-PCR is very challenging. Therefore, using a dissection method that provides a spatial resolution between manual dissection and LMC could be more efficient. In combination with real-time RT-PCR this approach may allow for the collection of larger data sets.

4.4. Conclusions

Our results demonstrate that the adult mouse heart exhibits a pattern of transmural expression gradients of Kv4 channel α-subunits and KChIP2(β-subunits) very similar to those in the ventricles of larger mammals. This suggests that homologous pathways may govern the development and maintenance of transmural gradients for repolarization. In the future it will be important to assess whether these expression gradients are maintained, or altered in the settings of cardiovascular disease (heart failure) [37] and/or hormonal imbalances (diabetes and hypothyroidism). Both are known to alter ventricular repolarization gradients and to elevate the risk of ventricular arrhythmias [38,39].

Previous studies have demonstrated a number of significant differences of fundamental electrophysiological mechanisms between adult mouse and human [40], and these differences should be kept in mind when comparing the two systems.

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References


