Published in final edited form as:

J Mol Cell Cardiol. 2008 June ; 44(6): 1002–1015. doi:10.1016/j.yjmcc.2008.03.023.

PPAR α -Mediated Remodeling of Repolarizing Voltage-Gated K⁺ (Kv) Channels in a Mouse Model of Metabolic Cardiomyopathy

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Abstract

Diabetes is associated with increased risk of diastolic dysfunction, heart failure, QT prolongation and rhythm disturbances independent of age, hypertension or coronary artery disease. Although these observations suggest electrical remodeling in the heart with diabetes, the relationship between the metabolic and the functional derangements is poorly understood. Exploiting a mouse model (MHC-PPAR α) with cardiac-specific overexpression of the peroxisome proliferator-activated receptor α (PPAR α), a key driver of diabetes-related lipid metabolic dysregulation, the experiments here were aimed at examining directly the link(s) between alterations in cardiac fatty acid metabolism and the functioning of repolarizing, voltage-gated K⁺ (Kv) channels. Electrophysiological experiments on left (LV) and right (RV) ventricular myocytes isolated from young (5–6 week) MHC-PPAR α mice revealed marked K⁺ current remodeling: I_{to,f} densities are significantly ($P < 0.01$) lower, whereas I_{ss} densities are significantly ($P < 0.001$) higher in MHC-PPAR α , compared with age-matched wild type (WT), LV and RV myocytes. Consistent with the observed reductions in I_{to,f} density, expression of the *KCNQ2* (Kv4.2) transcript is significantly ($P < 0.001$) lower in MHC-PPAR α , compared with WT, ventricles. Western blot analyses revealed that expression of the Kv accessory protein, KChIP2, is also reduced in MHC-PPAR α ventricles in parallel with the decrease in Kv4.2. Although the properties of the endogenous and the “augmented” I_{ss} suggest a role(s) for two pore domain K⁺ channel (K2P) pore forming subunits, the expression levels of *KCNK2* (TREK1), *KCNK3* (TASK1) and *KCNK5* (TASK2) in MHC-PPAR α and WT ventricles are not significantly different. The molecular mechanisms underlying I_{to,f} and I_{ss} remodeling in MHC-PPAR α ventricular myocytes, therefore, are distinct.

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Keywords

fatty acid metabolism; glucose metabolism; diabetes; diabetic cardiomyopathy; cardiac remodeling; Kv currents; repolarization; arrhythmias

Introduction

Although both fatty acid and glucose metabolism serve as fuel sources for the adult heart, fatty acids are the primary energy substrate. Nevertheless, cardiac muscle exhibits remarkable metabolic flexibility, switching between reliance on fatty acids and glucose, depending on nutritional and physiological status [1]. This metabolic flexibility is lost, however, in a number of pathological states, such as in myocardial hypertrophy and ischemia, and in diabetes [2–5]. The diabetic heart, for example, relies almost exclusively on fatty acids to generate energy [3], whereas in the hypertrophied heart, glucose transport and utilization predominate [4,5]. Importantly, these derangements in substrate metabolism are associated with cardiac dysfunction. Diabetic cardiomyopathy, defined as heart failure in the context of diabetes in the absence of congenital heart disease, atherosclerosis or hypertension, for example, is associated with significant morbidity and mortality [5,6]. In addition, QT prolongation, a marker of altered ventricular repolarization, is prevalent in diabetes and is an independent risk factor for sudden cardiac death [7]. The relationships between derangements in cardiac metabolism and alterations in the electrical and mechanical functioning of the heart, however, are poorly understood.

Cardiac myocyte fatty acid transport and utilization pathways are controlled, in part, at the gene regulatory level [4,8]. The peroxisome proliferator-activated receptor alpha (PPAR α), a ligand-activated transcription factor, in particular, plays a critical role in the regulation of genes involved in myocardial fatty acid and energy metabolism, and PPAR α activity is altered in a variety of myocardial disease states [8]. PPAR α expression and activity, for example, are elevated in diabetes and reduced in both cardiac ischemia and hypertrophy [8]. These pathologies are also associated with increased electrical instability and arrhythmia risk [9,10], suggesting the interesting possibility that alterations in cardiac metabolism are linked directly to changes in myocardial membrane excitability.

In a previously described transgenic mouse model (MHC-PPAR α) in which PPAR α is overexpressed in the postnatal heart driven by the α -myosin heavy chain promoter, fatty acid oxidation rates are increased and glucose uptake and metabolism are decreased, a metabolic phenotype that is remarkably similar to the diabetic heart [11,12]. Older MHC-PPAR α mice also exhibit features of diabetic cardiomyopathy, including activation of gene markers of pathologic hypertrophic growth and alterations in systolic and diastolic function [11]. In addition, the MHC-PPAR α phenotype is worsened in animals maintained on a high fat diet [12]. In contrast, fatty acid oxidation rates are reduced [13] and glucose oxidation and glycolysis rates are increased [14] in the hearts of mice (PPAR α –/–) lacking PPAR α [15,16].

Exploiting the MHC-PPAR α mouse model, the experiments here were designed to test the hypothesis that PPAR α -driven alterations in myocardial fatty acid uptake and metabolism, in the absence of any systemic metabolic (or other) disease, affect the expression, properties and/or functioning of the K⁺ channels that underlie ventricular repolarization. The results presented demonstrate that alterations in myocardial metabolism result directly in the remodeling of repolarizing K⁺ channels, changes that would be expected to impact cardiac functioning and arrhythmia susceptibility.

Materials and Methods

Animals were handled in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health), and the protocols used were approved by the Animal Studies Committee at Washington University Medical School. The MHC-PPAR α [11] and PPAR α -/- [13] mice have been described previously.

Electrophysiological Recordings

In most of the experiments here, whole-cell patch clamp recordings were obtained from myocytes isolated from the left (LV) and right (RV) ventricles of adult (5–6 week) wild type (WT; $n = 9$) and MHC-PPAR α ($n = 9$) C57BL6 [11] mouse hearts by enzymatic dissociation and mechanical dispersion using previously described methods [17,18]. Electrophysiological experiments were completed on both RV and LV myocytes because previous studies have revealed marked (~2 fold) differences in O₂ consumption between the right and left ventricles [19,20], raising the interesting possibility that there might be regional differences in the functional impact of alterations in PPAR α expression. Experiments were also performed on RV and LV cells isolated from older (12–14 week) MHC-PPAR α ($n = 5$) animals in which left ventricular hypertrophy and altered cardiac mechanical functioning have been demonstrated [11]. Recordings were also obtained from LV and RV cells isolated from (Sv129) animals (PPAR α -/-) in which the PPAR α locus was disrupted by homologous recombination [15]. In these experiments, cells from young (5–6 week old) PPAR-/- animals ($n = 6$) were compared to cells from age-matched WT Sv129 littermates ($n = 5$).

Electrophysiological experiments were conducted using an Axopatch 1D amplifier (Axon Instruments), interfaced to a Dell microcomputer with a Digidata 1322 Series analog/digital interface (Axon), using pClamp 8 or 9 (Axon). Whole-cell voltage-clamp experiments were conducted within 10 hours of myocyte isolation at room temperature (22–24°C). Recording pipettes contained (in mmol/L): 135 KCl, 1 MgCl₂, 10 EGTA, 10 HEPES and 5 glucose (pH 7.2; 310 mOsm). The bath solution contained (in mmol/L): 136 NaCl, 4 KCl, 1 CaCl₂, 2 MgCl₂, 5 CoCl₂, 10 HEPES, 0.02 tetrodotoxin (TTX) and 10 glucose (pH 7.4; 300 mOsm). For current-clamp experiments, the TTX and CoCl₂ were omitted from the bath, and action potentials were recorded in response to brief (1–2 ms) depolarizing current injections at 1 Hz.

Whole-cell membrane capacitances and series resistances were compensated electronically prior to recording voltage-clamp currents. Voltage errors resulting from the uncompensated series resistance were always ≤ 8 mV and were not corrected. Experimental data were acquired at variable sampling frequencies; current and voltage signals were filtered on-line at 5 kHz prior to digitization and storage. Depolarization-activated outward K⁺ (K_v) currents were routinely recorded in response to 4.5 s voltage steps to potentials between -40 and +40 mV from a holding potential (HP) of -70 mV; voltage steps were presented in 10 mV increments at 15 s intervals. Inwardly rectifying K⁺ currents, I_{K1}, were recorded in response to 350 ms voltage steps to test potentials between -90 and -120 mV (in 10 mV increments) from the same HP.

Pharmacological characterization of the steady-state non-inactivating component of the total K_v current, I_{ss}, was performed by examining the effects of brief extracellular applications of quinidine (100 μ M), halothane (200 μ M), riluzole (100 μ M), Zn²⁺ (200 μ M), Gd³⁺ (100 μ M), anandamide (10 μ M) or CHCl₃ (1 mM). In addition, the effects of the acidification of the (extracellular) bath solution (pH_e $\approx$ 6.5) were examined. All compounds were obtained from Sigma Chemical Co. (St. Louis, MO), and all drug-containing bath solutions were prepared immediately before use. In these experiments, I_{ss} was recorded during 10 s voltage steps to potentials between -50 and +50 mV, presented at 20 s intervals in 10 mV increments.

Electrocardiographic Recordings

Surface electrocardiographic (ECG) recordings were obtained from anesthetized (avertin; intraperitoneal injection) 5–6 week old WT (n = 16), MHC-PPAR α (n = 13) and PPAR α -/- (n = 8) animals. For analysis of ECGs, the onsets and offsets of the P, Q, R, S, and T waves were determined by measuring the earliest (onset) and the latest (offset) times from the lead II. Measured QT intervals were also corrected for differences in heart rate using the formula $QT_c = QT / (RR/100)^{1/2}$, as previously described by Mitchell and colleagues [21].

RNA Preparation and SYBR Green Quantitative RT-PCR

RNA was isolated and analyzed using standard methods previously described in detail [22, 23]. Briefly, hearts were rapidly excised from 5–6 week old WT (n = 6) and MHC-PPAR α (n = 6) mice following sacrifice by cervical dislocation. The left ventricles were dissected from each heart and flash-frozen (separately) in liquid nitrogen for further RNA isolation. Total RNA was isolated from each sample and DNase treated using the RNeasy Fibrous Tissue Mini Kit (Qiagen). The quality of RNA in every sample was examined by gel electrophoresis. Genomic DNA contamination was assessed by PCR amplification of each of the total RNA samples without prior cDNA synthesis; no genomic DNA was detected.

First strand cDNA was synthesized from 2 μ g of total RNA (from each left ventricular sample) using the High-Capacity cDNA Archive Kit (Applied Biosystems). The expression levels of genes encoding several K⁺ channel subunits, including Kv4.2, Kv4.3, KChIP2, Kv β 1, Kv1.5, Kv2.1, TREK1, TASK1, TASK2, TASK3, Kir2.1, and Kir2.2, as well as atrial natriuretic factor (ANF) and β myosin heavy chain (β MHC), markers of cardiac hypertrophy [24], were determined by quantitative RT-PCR using 1X SYBR Green PCR Master Mix (Applied Biosystems). PCR reactions were performed on 10 ng of cDNA using the following sequence specific primer pairs on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems).

Primer Pairs Used for SYBR Green Quantitative RT-PCR

Kv1.5	(<i>KCNK5</i>)	5'-CCTGCGAAGGTCTCTGTATGC 5'-TGCCTCGATCTCTCTTTACAAATCT
Kv2.1	(<i>KCNB1</i>)	5'-CACACAGCAATAGCGTTCAACTT 5'-AGGCGTAGACACAGTTCGGC
Kv4.2	(<i>KCND2</i>)	5'-GCCCGAGCACCTAGTCGTT 5'-CACCACGTCGATGATACTCATGA
Kv4.3	(<i>KCND3</i>)	5'-CCTAGCTCCAGCGGACAAGA 5'-CCACTTACGTTGAGGACGATCA
KChIP2	(<i>KCNIP2</i>)	5'-GGCTGTATCAGGAAGGAGGAA 5'-CCGTCCCTTGTCTGTCCATC
Kv β 1	(<i>KCNAB1</i>)	5'-AAATACCCAGAAAGGCAAGTGT 5'-ATCTAGCATGTGCCGAGGAA
TASK1	(<i>KCNK3</i>)	5'-GCTTCCGCAACGTCTATGC 5'-GGGATGGAGTACTGCAGCTTCT
TASK2	(<i>KCNK5</i>)	5'-TCAGATCACGTGTACAGCCATCT 5'-CATTCTTCTGTACCATGAACACA
TASK3	(<i>KCNK9</i>)	5'-GACGTGCTGAGGAACACCTACTT 5'-GGTGTGCATTCCAGGAGGAA
TREK1	(<i>KCNK2</i>)	5'-CCTTTTGTGCCAGACTGTTTC 5'-GCAAAGCATTGAGATTCTCATAG
Kir2.1	(<i>KCNJ2</i>)	5'-AAGAGCCACCTGTGGAAGCT 5'-CCTTCTGAAGTGATCCTAGATTGAGA
Kir2.2	(<i>KCNJ12</i>)	5'-AGCACCACCCTGACCACAA 5'-CTGAGCAACCTTACCCAA
Nav1.5	(<i>SCN5A</i>)	5'-AGTGCCACCAATGCCTTGATC 5'-GCGGAGGGTCGTGTTGTG
Cx40	(<i>GJA5</i>)	5'-CCAAACCAGGAGCAGATTCC 5'-GCGTACTCTGGCTTCTGGCTAT
Cx43	(<i>GJA1</i>)	5'-ACAAGTCTTCCCCATCTCTCA 5'-GTGTGGGCACAGACAGAAT
Cx45	(<i>GJA7</i>)	5'-ACAGTAAAAGGAGGGAACCTTGATGA 5'-GGGTGTGTTCCAAGTGAAAGG

Primer Pairs Used for SYBR Green Quantitative RT-PCR

ANF	(<i>NPPA</i>)	5'-CACTGTGGCGTGGTGAACA 5'-TCGTGATAGATGAAGGCAGGAA
β MHC	(<i>MYH7</i>)	5'-TGAATGAGCACCGGAGCAA 5'-CTGGCTGGTGAGGTCATTGA
HPRT	(<i>HPRT</i>)	5'-TGAATCACGTTTGTGTCATTAGTGA 5'-TTCAACTTGCCTCATCTTAGG

The cycling conditions included a hot start at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All primer pairs were tested using mouse cDNA as template, and PCR primer pairs giving 90–100% efficacy were chosen. In all cases, a single amplicon of the appropriate melting temperature or size was detected using the dissociation curve or gel electrophoresis, respectively. Results for each sample (mouse) were normalized to *HPRT*, and expressed according to the $2^{-\Delta C}$ method [25], as relative mRNA expression compared with *HPRT*. Negative control experiments using RNA samples incubated without reverse transcriptase during cDNA synthesis showed no amplification.

Western Blot Analyses

Western blot analyses were performed on fractionated ventricular membrane proteins prepared from adult (5–6 week) WT (n = 10) and MHC-PPARα (n = 10) mice using previously described methods [26,27]. The polyclonal rabbit anti-Kv4.2 antibody (Chemicon, Temecula, CA) and the monoclonal anti-KChIP2 antibody (UC Davis NINDS/NIMH Neuromab Facility, Davis, CA) have been tested previously for specificity and cross reactivity [26,27]. Commercially available polyclonal rabbit anti-connexin 43 (Zymed Laboratories, Invitrogen, Carlsbad, CA) and rabbit anti-connexin 45 (generously provided by Dr. Thomas Steinberg, Washington University Medical School) were used at (1:5000) dilutions suggested by the suppliers. The polyclonal rabbit anti-TASK1 and anti-TASK2 antibodies (Alomone, Jerusalem, Israel) were tested for specificity as per the recommendations of the supplier against brain and kidney extracts. Equal amounts of proteins (40 μg) were fractionated on (7.5% or 10 %) SDS-PAGE gels and transferred to PVDF membranes. Equal protein loading was checked by Ponceau staining. Membranes were then blocked, and incubated overnight at 4°C with primary antibodies appropriately detailed in buffer. Following washing, the membranes were incubated with goat anti-rabbit or goat anti-mouse horseradish peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL).

Membranes were incubated with the SuperSignal West Dura Extended Duration substrate (Pierce), and signal was exposed to x-ray film (Kodak, Rochester, NY). In these experiments, the membranes probed with anti-Kv subunit-specific antibodies were subsequently stripped (2% SDS, 0.7% β-mercaptoethanol) and re-probed with a monoclonal anti-transferrin receptor antibody (Sigma Chemical Company, St. Louis, MO) to ensure equal protein loading of the different lanes. In some experiments, the K⁺ channel subunit specific anti-body and the anti-transferrin receptor antibody were combined and membranes were probed with both antibodies in a single step. Films from individual experiments were scanned, and the densities of specific bands were determined. In each group, results are expressed as relative K⁺ channel subunit protein expression level *versus* the endogenous control protein transferrin receptor.

Data Analysis

Voltage-clamp data were compiled and analyzed using *Clampfit* (Axon Instrument) and *GraphPad* (Prism) software. Integration of the capacitive transients recorded during brief ± 10 mV voltage steps from the HP (−70 mV) allowed determination of whole-cell membrane

capacitances (C_m). Leak currents were always <10 pA and were not corrected. Peak K_v currents at each test potential were defined as the maximal outward K^+ current recorded during the 4.5 s voltage steps. The decay phases of the outward K^+ currents evoked during 4.5 s depolarizing voltage steps in all LV and RV cells were fitted by a double exponential function of the form: $y(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + B$, where t is time, τ_1 and τ_2 are the decay time constants, A_1 and A_2 are the amplitudes of the inactivating current components ($I_{to,fast}$ or $I_{to,f}$ and $I_{K,slow}$), and B is the amplitude of the non-inactivating current component, I_{ss} [17,18]. In the pharmacological experiments described, I_{ss} densities were measured directly as the currents remaining at the end of 10 s depolarizing voltage steps. In all cells, I_{K1} densities were determined from the amplitudes of the currents measured at the end of 350 ms hyperpolarizing voltage steps (-90 to -130 mV) from a HP of -70 mV.

Current amplitudes in each cell were normalized to whole-cell membrane capacitances (in the same cell), and current densities (pA/pF) are reported. Resting membrane potentials (RMP), action potential amplitudes (APA) and action potential durations at 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) repolarization were measured using *Clampfit*. All data are presented as means $\pm$ S.E.M. The statistical significance of observed differences between the various experimental groups (of animals or cells) was evaluated using the Student's *t* test, the one-way analysis of variance (ANOVA) or the Student-Newman-Keuls test; *P* values are presented in the text.

Results

Remodeling of Ventricular K^+ Currents with Alterations in Myocardial PPAR α Expression

To determine the effects of PPAR α -driven alterations in myocardial lipid metabolism on repolarizing voltage-gated K^+ (K_v) currents, whole-cell K_v currents were recorded from ventricular myocytes isolated from MHC-PPAR α (C57BL6) mice [11] and compared with the currents in myocytes from wild type (WT) littermate controls. Because previous studies have demonstrated age-dependent changes in cardiac structure and functioning in MHC-PPAR α transgenics [11], initial experiments were performed on young adult (5–6 week old) MHC-PPAR α animals in which no pathology is evident [11]. As illustrated in Figure 1A, peak outward K_v current (I_{peak}) densities are similar in left ventricular (LV) myocytes isolated from (5–6 week) WT and MHC-PPAR α animals, although the waveforms of the currents are distinct. Quantitative analyses confirmed that mean $\pm$ SEM I_{peak} densities in MHC-PPAR α (62.9 ± 3.5 pA/pF; $n = 25$) and WT (69.0 ± 3.6 pA/pF; $n = 19$) LV myocytes are not significantly different (Figure 1B). Mean $\pm$ SEM I_{peak} densities in myocytes isolated from the right ventricles (RV) of (5–6 week) WT and MHC-PPAR α animals are also quite similar (Table 1).

The decay phases of the K_v currents in adult WT mouse LV (and RV) myocytes are best described by the sum of two exponentials, reflecting the fast transient K_v current, $I_{to,f}$, the slow delayed rectifier current, $I_{K,slow}$, and a non-inactivating current, I_{ss} [17,18]. The decay phases of the K_v currents in MHC-PPAR α LV (and RV) myocytes were also well described by two exponentials with inactivation time constants for $I_{to,f}$ and $I_{K,slow}$ that are not measurably different from those in WT cells (Table 1). Kinetic analyses of the decay phases of the currents, however, revealed that mean $\pm$ SEM $I_{to,f}$ densities are significantly ($P < 0.01$) lower and that mean $\pm$ SEM I_{ss} densities are significantly ($P < 0.001$) higher in MHC-PPAR α , compared with WT, LV and RV myocytes (Figure 1B; Table 1). The differences in $I_{to,f}$ (and I_{ss}) densities do not reflect differences in cell sizes, as mean $\pm$ SEM whole-cell membrane capacitances (C_m) in WT (136 ± 7 pF; $n = 21$) and MHC-PPAR α (123 ± 5 pF; $n = 19$) RV cells are similar. Mean $\pm$ SEM C_m values in WT (155 ± 8 ; $n = 19$) and MHC-PPAR α (152 ± 8 pF; $n = 25$) LV cells are also not significantly different. In contrast to $I_{to,f}$ and I_{ss} , there are no significant differences in $I_{K,slow}$ densities in LV (or RV) myocytes from MHC-PPAR α and WT animals (Table 1). The (mean $\pm$ SEM) densities of the inward rectifying K^+ (K_{ir}) current, I_{K1} , in MHC-PPAR α

LV and RV myocytes, however, are significantly ($P < 0.001$) higher than in WT LV and RV cells (Table 1).

As illustrated in Figure 2A, action potential waveforms in LV cells isolated from 5–6 week old WT and MHC-PPAR α animals are similar. No significant differences in action potential durations at 20 (APD₂₀), 50 (APD₅₀) or 90 (APD₉₀) percent repolarization were evident (Figure 2B). Mean $\pm$ SEM APD₉₀ values in WT and MHC-PPAR α LV myocytes, for example, were 18.2 ± 1.3 ms ($n = 10$) and 19.3 ± 1.8 ms ($n = 18$), respectively. These observations suggest that the reciprocal changes in $I_{to,f}$ and I_{ss} , as well perhaps as the augmentation of I_{K1} , function to normalize ventricular action potential durations in (5–6 week) MHC-PPAR α animals. Unexpectedly, analyses of surface ECG recordings revealed significantly ($P < 0.001$) slower heart rates and longer QT intervals in MHC-PPAR α , compared with WT, animals (Table 2). Following normalization for differences in heart rates, however, there are no significant differences in corrected QT (QT_c) intervals in WT and MHC-PPAR α animals. *Nevertheless, the finding of bradycardia in (5–6 week) MHC-PPAR α animals clearly suggests that myocardial overexpression of PPAR α impacts pacemaker function. The ECG analyses also revealed significant ($P < 0.001$) prolongation of QRS durations in (5–6 week) MHC-PPAR α , compared with WT, animals, suggesting altered ventricular conduction velocity (see Discussion).*

To determine if reciprocal changes in K_v and/or K_{ir} currents result from the loss of PPAR α , LV and RV myocytes were also isolated from mice (Sv129/PPAR α –/–) with a targeted disruption in the PPAR α locus [13,15,16]. As illustrated in Figure 3, peak K_v current densities are higher in PPAR α –/–, compared with WT (Sv129), LV myocytes. Kinetic analyses of the currents revealed that increased $I_{to,f}$ density underlies the observed increase in peak K_v currents in PPAR α –/– (compared with WT) LV myocytes (Table 1). Similar results were obtained in experiments on PPAR α –/– (and WT) RV myocytes (Table 1). The higher $I_{to,f}$ densities in PPAR α –/– ventricular myocytes do not reflect differences in cell sizes: mean $\pm$ SEM whole-cell C_m values in WT (141 ± 5 pF; $n = 29$ and 104 ± 4 pF; $n = 28$) and PPAR α –/– (136 ± 4 pF; $n = 36$; 131 ± 8 pF; $n = 25$) Sv129 LV and RV myocytes are not significantly different. Although $I_{to,f}$ densities are higher in PPAR α –/–, than in WT, LV and RV myocytes, the time- and voltage-dependent properties of the currents are indistinguishable (Table 1). In addition, and in contrast to the findings in MHC-PPAR α LV and RV cells, there were no measurable differences in I_{ss} or I_{K1} densities in PPAR α –/–, compared with WT, LV or RV myocytes (Table 1).

Molecular Basis of K_v Current Remodeling in MHC-PPAR α Ventricles

Previous studies have demonstrated that mouse ventricular $I_{to,f}$ is encoded by poreforming (α) subunits of the K_v4 subfamily [28], and that $I_{K,slow}$ reflects two molecularly distinct components, produced by $K_v2.1$ and $K_v1.5$ [29–31]. Biochemical data suggest that mouse ventricular $I_{to,f}$ channels are heteromeric, comprising the $K_v4.2$ and $K_v4.3$ α subunits, as well as the K_v channel accessory subunits, $KChIP2$ and $K_v\beta1$ [26,27,32]. To explore the molecular basis of $I_{to,f}$ remodeling in MHC-PPAR α ventricular myocytes (Figure 1; Table 1), the expression levels of the transcripts encoding $K_v4.2$, $K_v4.3$, $KChIP2$ and $K_v\beta1$ in (5–6 week) WT and MHC-PPAR α ventricles were determined using SYBR Green quantitative RT-PCR. The transcript expression levels of $KCNJ2$ and $KCNJ12$, which encode the I_{K1} channel subunits $Kir2.1$ and $Kir2.2$ [33], and the $KCNK$ transcripts $KCNK2$, $KCNK3$, $KCNK5$, which encode the two pore domain K^+ (K2P) channel α subunits, $TREK1$, $TASK1$ and $TASK2$ [34–40], were also examined. Previous studies have suggested roles for both $TREK1$ and $TASK1$ in the generation of steady-state (non-inactivating) repolarizing K^+ currents in rodent atrial and ventricular myocytes [34–38]. Although $KCNK9$ ($TASK3$) expression was also examined, this transcript was barely detectable in WT and in MHC-PPAR α ventricles (and no data, therefore, are presented for $KCNK9$ ($TASK3$) expression in Figure 4).

These analyses revealed that *KCND2* (Kv4.2) expression is reduced markedly in MHC-PPAR α , compared with WT, ventricles (Figure 4). The expression levels of the other components of $I_{to,f}$, *KCND3* (Kv4.3), *KCHIP2* (KChIP2) and *KCNAB1* (Kv β 1), however, are not significantly different in MHC-PPAR α and WT ventricles (Figure 4). These experiments also revealed no significant differences in the expression levels of the K2P channel subunits, *KCNK2* (TREK1), *KCNK3* (TASK1) or *KCNK5* (TASK2) in WT and MHC-PPAR α ventricles (Figure 4). The expression levels of *KCNJ2* (Kir2.1) and *KCNJ12* (Kir2.2) are also similar in WT and MHC-PPAR α ventricles (Figure 4). Quantitative RT-PCR using primer pairs for atrial natriuretic factor (ANF) and β myosin heavy chain (β MHC), both of which are markers of cardiac hypertrophy [24], was also completed. No significant differences in ANF or β MHC expression were observed (Figure 4), consistent with previous reports [11] that hypertrophy is not evident in young (5–6 week old) MHC-PPAR α animals (see Discussion).

Western blot analysis revealed markedly reduced Kv4.2 protein expression (Figure 5A) in (5–6 week) MHC-PPAR α , compared with WT, ventricles. On average, Kv4.2 expression in MHC-PPAR α ventricles was $7 \pm 3\%$ ($n = 10$) of the WT protein expression level (Figure 5H). The reduction in Kv4.2 protein expression ($\sim 90\%$) is larger than might have been predicted based on the observed ($\sim 50\%$) reduction in *KCND2* transcript (Figure 4) expression. In parallel with the decrease in Kv4.2, KChIP2 protein expression (Figure 5B) was also reduced in MHC-PPAR α ventricles to $19 \pm 8\%$ ($n = 10$) of the WT level (Figure 5H), whereas there were no detectable differences in Kv4.3 protein expression in WT and MHC-PPAR α ventricles (not illustrated). Although there were animal to animal variations in Kv4.2 (Figure 5A) and KChIP2 (Figure 5B) protein expression in MHC-PPAR α ventricles, these differences do not reflect technical problems, such as variability among samples in the efficiency of protein extraction and/or gel loading, as evidenced by the fact that there were no significant animal to animal differences evident in Western blots of the same protein samples probed with an anti-transferrin receptor antibody (Figure 5G). Similar results were obtained when the Kv subunit probed Westerns were stripped and re-probed with the anti-transferrin receptor antibody (not illustrated). Consistent with the similarities in transcript expression levels (Figure 4), Western blots of fractionated ventricular proteins revealed no significant differences in the expression levels of the TASK1 (Figure 5C) and/or TASK2 (Figure 5D) proteins (Figure 5H) in WT and MHC-PPAR α ventricles. Marked reductions in connexin 43 (Cx43; Figure 5E), but not in connexin 45 (Cx45; Figure 5F), expression were evident in MHC-PPAR α , compared with WT, ventricles (Figure 5H). Similar to the findings with *KCND2* (Kv4.2), the ($\sim 90\%$) decrease in Cx43 protein expression is larger than might have been expected based on the observed ($\sim 50\%$) decrease in *GJA1* (Cx43) message (Figure 4) expression (see Discussion).

Distinct Properties of I_{ss} in WT and MHC-PPAR α Ventricular Myocytes

As illustrated in Figure 1, I_{ss} densities are higher in (5–6 week) MHC-PPAR α , compared with WT, LV and RV cells. In both WT and MHC-PPAR α LV myocytes, I_{ss} is outwardly rectifying and the voltage-dependent properties of the currents are similar (Figure 6A). Analysis of the “up-regulated” component of the (I_{ss}) current in (5–6 week) MHC-PPAR α LV cells, however, revealed a non-inactivating current in MHC-PPAR α cells with a nearly linear current/voltage relationship (Figure 6B). The voltage-dependences of the endogenous and the augmented I_{ss} , therefore, are distinct (Figure 6). The nearly linear current voltage-relation for the up-regulated I_{ss} , however, also suggests a role for K2P channel α subunits [39,40]. Previous studies have shown that heterologous expression of K2P α subunits gives rise to non-inactivating K^+ currents with linear or weakly rectifying current-voltage relations [39]. In addition, as noted above, a role for K2P subunits in the generation of non-inactivating K^+ currents in rodent myocytes has been suggested previously [34–38]. To explore this hypothesis further, experiments exploiting several blockers of K2P channels were performed to compare the pharmacological properties of the endogenous and the augmented I_{ss} .

In whole-cell voltage-clamp experiments, the effects of brief (2 min) bath applications of a number of putative K2P channel blockers, including zinc (Zn^{2+}), gadolinium (Gd^{3+}), anandamine, riluzole, halothane and chloroform (CHCl_3) [39] on I_{ss} in WT and MHC-PPAR α LV myocytes were determined. These experiments revealed that several of the blockers tested differentially affected I_{ss} in MHC-PPAR α LV myocytes (Figure 6C). I_{ss} in MHC-PPAR α cells, for example, was blocked preferentially by 100 μM Gd^{3+} ($20 \pm 4\%$, $n = 12$), by 200 μM Zn^{2+} ($20 \pm 3\%$, $n = 6$), by 10 μM anandamide ($30 \pm 6\%$, $n = 10$) and by 1mM CHCl_3 ($33 \pm 7\%$, $n = 7$) (Figure 6C). In contrast, none of these agents appreciably affected I_{ss} in WT myocytes (Figure 6C). Together, these observations suggest that the augmented I_{ss} in MHC-PPAR α ventricular myocytes is indeed distinct from I_{ss} in WT cells. The pharmacological profile of the currents (Figure 6C) further suggests that TASK1- or TASK2-encoded K2P channels likely underlie the augmented I_{ss} in MHC-PPAR α ventricular myocytes (see Discussion).

Age-Related Remodeling of Kv Currents in MHC-PPAR α Ventricular Myocytes

Left ventricular hypertrophy (LVH) develops with aging in adult MHC-PPAR α mice [11]. To determine if the age-related development of LVH is associated with further Kv current remodeling, experiments were also performed on LV myocytes isolated from older (12–14 week) MHC-PPAR α (and WT) mice. As illustrated in Figure 7, these experiments revealed marked differences in the Kv currents in LV myocytes from 12–14 week MHC-PPAR α , compared with age-matched WT, animals. Peak Kv current densities, for example, are significantly ($P < 0.001$) lower in (12–14 week) MHC-PPAR α , compared with WT, LV cells (Figure 7B; Table 3). In addition, the Kv currents in LV cells from 12–14 week old MHC-PPAR α animals (Figure 7) are distinct from those measured in LV cells from 5–6 week old MHC-PPAR α animals (Figure 1).

Analyses of the Kv currents revealed that $I_{to,f}$ and $I_{K,slow}$ densities are significantly ($P < 0.001$) lower in MHC-PPAR α , compared to WT, LV myocytes from 12–14 week animals (Figure 7B; Table 3). Similar to the findings in cells from 5–6 week animals, however, I_{ss} densities are significantly ($P < 0.001$) higher in (12–14 week) MHC-PPAR α , compared with WT, LV myocytes (Figure 7B; Table 3). Importantly, peak Kv current densities, as well as the densities (and properties) of $I_{to,f}$, $I_{K,slow}$ and I_{ss} in LV myocytes isolated from 12–14 week WT animals (Figure 7; Table 3) are not significantly different from those in LV cells from 5–6 week WT animals (Figure 1; Table 1). No age-related remodeling of Kv currents is evident, therefore, in WT ventricular cells.

In contrast to the findings in LV myocytes, the densities and properties of $I_{to,f}$ and $I_{K,slow}$ in RV myocytes from 12–14 week MHC-PPAR α animals (Table 3) are similar to those in RV cells from young (5–6 week) animals (Table 1). Similar to the findings in cells from younger animals (Table 1), for example, I_{ss} densities are significantly higher in RV myocytes from 12–14 week MHC-PPAR α animals, compared with I_{ss} densities in age-matched WT RV cells (Table 3). The selective attenuation of $I_{to,f}$ and $I_{K,slow}$ in LV myocytes from 12–14 week MHC-PPAR α mice suggests that Kv current remodeling likely occurs secondary to the development of the left ventricular hypertrophy previously described in older MHC-PPAR α animals [11], rather than age-related differences in metabolic regulation (see Discussion).

Discussion

Remodeling of Kv Currents in MHC-PPAR α Ventricular Myocytes

The experiments here demonstrate that chronic, cardiac specific activation of PPAR α , a key regulator of fatty acid and glucose uptake and metabolism [11] results in ventricular Kv (and Kir) current remodeling. Specifically, $I_{to,f}$ densities are decreased and I_{ss} densities are increased

in LV and RV myocytes from 5–6 week old MHC-PPAR α mice; I_{K1} densities are also increased in MHC-PPAR α ventricular cells. Although it has been shown previously that attenuation of $I_{to,f}$ results in action potential prolongation [28,32,41], action potential waveforms in MHC-PPAR α and WT cells are not significantly different. The simplest interpretation of these results is that the increases in I_{ss} and I_{K1} (Table 1) compensate for decreased $I_{to,f}$ in MHC-PPAR α ventricular myocytes, resulting in action potentials indistinguishable from those recorded in WT cells.

Surface ECG recordings revealed significantly ($P < 0.001$) longer QT and QRS intervals, as well as slower heart rates, in young (5–6 week) MHC-PPAR α , compared with WT, animals. Normalization for the differences in heart rates revealed that corrected QT (QT_c) intervals in WT and MHC-PPAR α animals are similar, consistent with the similarities in ventricular action potential durations. The prolongation of QRS durations observed in (5–6 week) MHC-PPAR α animals, however, suggests that ventricular conduction is affected by myocardial overexpression of PPAR α . *Alterations in ventricular conduction velocities were also observed with (tamoxifen) induced Cre-recombinase mediated deletion of the Cx43 locus in (adult) mice [42], suggesting a role for reduced Cx43 expression in the ECG abnormalities observed here in MHC-PPAR α animals. Although ~50% reductions in Cx43 expression in induced adult mice did not measurably affect conduction, Cx43 protein losses of 70 – 95 % resulted in marked slowing of conduction and, in addition, increased the dispersion of ventricular conduction and the propensity for ventricular arrhythmias [42]. The molecular/biochemical analyses here revealed that Cx43 protein expression is reduced by ~ 90% in (5–6 week) MHC-PPAR α , compared with WT, ventricles, suggesting that increased arrhythmia susceptibility may also be evident in MHC-PPAR α mice, a hypothesis that warrants direct testing. Interestingly, van Rijen and colleagues also reported that the results of immunoblot and immunohistochemical experiments did not reveal a correlation between residual Cx43 protein expression and arrhythmia susceptibility, suggesting that heterogeneities in the distribution of Cx43 likely play an important role in arrhythmogenesis in their model. Further experiments focused on examining Cx43 distribution in MHC-PPAR α ventricles will also be of interest.*

In contrast to the electrical remodeling in MHC-PPAR α myocytes, peak Kv current and $I_{to,f}$ densities are significantly ($P < 0.01$) higher in PPAR α -/- LV and RV myocytes compared with age- (and strain-) matched WT RV and LV cells. Interestingly, however, I_{ss} and I_{K1} densities are not affected by the targeted deletion of PPAR α . Taken together, these results suggest a role for PPAR α in the transcriptional regulation of $I_{to,f}$, but not of I_{ss} or I_{K1} (see also below). The experiments here also revealed no measurable effects on $I_{K,slow}$ densities or properties in LV or RV myocytes isolated from (5–6 week old) MHC-PPAR α (or PPAR α -/-) animals. These results were surprising in light of previous findings that $I_{K,slow}$ is selectively attenuated in ventricular myocytes isolated from transgenic animals (MHC-FATP) overexpressing the fatty acid transport protein in the heart [43]. Similar to the MHC-PPAR α transgenics [11], fatty acid accumulation and utilization are increased and glucose uptake and metabolism are decreased in MHC-FATP ventricles [43]. The MHC-FATP mice also exhibit features of diabetic cardiomyopathy including diastolic dysfunction and QT prolongation [43].

The distinct effects on myocardial Kv currents of these (seemingly) similar metabolic perturbations may suggest that there are differences in the specific metabolites that are accumulated (or depleted) in these two models and in the functional roles of these metabolites. Alternatively, there may be different transcriptional/post-transcriptional pathways activated in the two models, resulting in modulation of distinct Kv currents. Detailed analyses of the metabolic and molecular remodeling that occurs in MHC-PPAR α [11] and MHC-FATP [43] hearts will be necessary to explore these hypotheses directly and to delineate the mechanisms

underlying ventricular Kv current remodeling in these two genetic models of metabolic cardiomyopathy in type 2 diabetes.

Age-Related Kv Current Remodeling in MHC-PPAR α Left Ventricles

Although Kv current densities and properties in LV and RV myocytes from 5–6 week and 12–14 week WT animals are indistinguishable, peak Kv current, $I_{to,f}$ and $I_{K,slow}$ densities are significantly ($P < 0.001$) lower in LV myocytes from 12–14 (compared with 5–6) week MHC-PPAR α animals. Kv current densities (and properties) in RV myocytes from the older (12–14 week) animals, however, are indistinguishable from those determined in cells from young (5–6 week old) animals. The apparent specificity of age-related Kv current remodeling for the LV suggests that the observed attenuation of peak Kv current, $I_{to,f}$ and $I_{K,slow}$ densities in older MHC-PPAR α LV cells reflects the development of left ventricular hypertrophy, which appears to occur in older animals secondary to the metabolic perturbations produced by the overexpression of PPAR α [11]. In contrast to the further (age-related) remodeling of $I_{to,f}$ and $I_{K,slow}$ in MHC-PPAR α LV cells, there are no measurable changes in I_{ss} densities, suggesting that the mechanism(s) regulating the remodeling of I_{ss} are unique to the metabolic derangements associated with the overexpression of PPAR α and unaffected by cardiac hypertrophy.

A Novel Component of I_{ss} is Upregulated in MHC-PPAR α Ventricular Myocytes

Although the Kv α subunits encoding most of the Kv currents in mouse ventricular myocytes have been identified [28–31,41], the molecular correlate(s) of mouse ventricular I_{ss} has not been determined. Several studies, however, suggest that K2P α subunits [39,40], such as TREK or TASK, encode steady-state outward K^+ currents in rodent cardiomyocytes [34–38]. The pharmacological experiments here demonstrate that the properties of I_{ss} in WT and MHC-PPAR α LV cells are distinct. The “up-regulated” I_{ss} in MHC-PPAR α myocytes, for example, is more sensitive than endogenous I_{ss} to Zn^{2+} and to anandamide, suggesting a role for TASK subunits, particularly TASK1 [36–38]. The quantitative RT-PCR analysis here demonstrates that the TASK1 transcript is abundant in adult mouse ventricles (see Figure 4). The molecular and biochemical studies here, however, also revealed no measurable differences in the expression of the TASK1 (or the TASK2) transcript/protein in WT and MHC-PPAR α ventricles. These observations suggest that, if TASK subunits indeed underlie mouse ventricular I_{ss} , the increased (I_{ss}) densities in MHC-PPAR α ventricles likely reflect alterations in the subunit composition of the channels, perhaps through the formation of K2P channel heterodimers [44] or associations with (yet to be identified) channel auxiliary subunits or regulatory proteins. Alternatively, posttranslational modifications of I_{ss} channel subunits and/or regulatory proteins may play a role in the upregulation of I_{ss} densities in MHC-PPAR α ventricular myocytes.

It has also been suggested that increased mitochondrial respiration can activate TASK-like currents in cardiac, and other, cells directly [45,46]. Specifically, it has been reported that increased fatty acid uptake and utilization, which will lead secondarily to increased mitochondrial activity, up-regulates steady state TASK-like K^+ currents [46]. It is of interest to note here that changes in I_{ss} expression have also been reported in other (rodent) diabetes models [47–49]. In a rat model of type 1 diabetes, for example, decreased ventricular I_{ss} densities have been observed, whereas in a rat model of type 2 diabetes, both increases and decreases in ventricular I_{ss} have been reported [47–49]. The results here demonstrate that cardiac-specific overexpression of PPAR α results directly in increased functional mouse ventricular I_{ss} densities.

I_{to,f} Remodeling in MHC-PPAR α Ventricles: Relation to Previous Studies

Previous studies have demonstrated decreased ventricular I_{to,f} densities and alterations in Kv4 α subunit expression [47,49,50] in animal models of both type 1 and 2 diabetes [47–52]. At least one previous report, however, failed to identify changes in I_{to,f} densities in genetic (rat and mouse) models of diabetes [53]. In addition, as noted above, I_{to,f} densities are unaffected in MHC-FATP mice that also exhibit characteristic features of diabetic cardiomyopathy [43]. Taken together, these results suggest that the MHC-PPAR α mouse model [11,12] used here, is a more robust and, therefore, a more useful genetic model for studies aimed at defining the mechanisms underlying cardiac remodeling due to metabolic derangements, including diabetes. It is interesting to note that it has been reported that exposure to polyunsaturated fatty acids, which are elevated in the membranes of MHC-PPAR α ventricular myocytes [11,12], results directly in decreased I_{to,f} densities [54,55]. The results presented here, however, revealed that *KCND2* (Kv4.2) message expression is reduced (by ~50%) in MHC-PPAR α ventricles, suggesting MHC-PPAR α -mediated transcriptional regulation of *KCND2* expression (and, therefore, I_{to,f} densities). *The loss (~90%) of Kv4.2 protein expression in MHC-PPAR α ventricles was substantially larger than the observed reduction (~50%) in the expression of the KCND2 transcript, suggesting that additional, post-transcriptional mechanisms contribute to the attenuation of I_{to,f}. Interestingly, a similar discrepancy between the reduction in Cx43 transcript (~50%) and protein (~90%) expression were observed, again suggesting roles for transcriptional and post-transcriptional mechanisms in Cx43 remodeling in MHC-PPAR α ventricles.*

*In contrast to the marked reduction in KCND2 transcript expression, there were no detectable differences in the expression levels of the transcripts encoding several other Kv subunits, Kv4.3, KChIP2, Kv β 1, that also contribute to I_{to,f} [26,27,32]. Western blot analyses, however, revealed that KChIP2 (as well as Kv4.2) protein expression is markedly (~90%) decreased in MHC-PPAR α ventricles. The loss of the KChIP2 protein in the absence of changes in the *KCNIP2* transcript is reminiscent of recent findings in *KCND2*^{−/−} animals, in which Kv4.2 was deleted by homologous recombination [23]. Although the underlying mechanism(s) responsible for the down-regulation of the KChIP2 protein remains to be delineated, the simplest interpretation of these combined experimental observations is that KChIP2 protein expression is reduced secondary to the down-regulation of Kv4.2 [23].*

In MHC-PPAR α ventricles, the increases in fatty acid uptake and utilization are accompanied by decreases in glucose transport and metabolism [11]. Interestingly, it has also been reported that inhibition of glucose oxidation in rabbit atria results in decreased I_{to} densities [56]. These observations suggest the interesting possibility that altered glucose oxidation may also play a direct role in regulating the functional expression of I_{to,f} channels [57]. Experiments focused on testing this hypothesis directly will provide new insights into the roles of fatty acid and glucose metabolism in the regulation of functional cardiac I_{to} channels and into the molecular mechanism involved in mediating these effects.

Acknowledgements

The authors wish to thank Ms. Teresa Leone, Ms. Amy Coleman and Mr. Rick Wilson for screening and maintenance of animals and for expert technical assistance throughout the course of these studies. The authors also thank Dr. Thomas Steinberg for the generous gift of the anti-Cx45 antibody. Finally, the authors acknowledge the financial support provided by the National Institutes of Health (R01HL-034161 and R01HL-066388 to JMN and P50 HL077113 to DPK) and the Heartland Affiliate of the American Heart Association (Postdoctoral Fellowships to FA and CM).

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Figure 1. $I_{to,f}$ densities are lower and I_{ss} densities are higher in MHC-PPAR α , than in WT, ventricular myocytes

(A) Depolarization-activated outward K^+ (K_v) currents in LV myocytes isolated from 5–6 week old WT and MHC-PPAR α animals were evoked in response to 4.5 s voltage steps to potentials between -40 and $+40$ mV from a holding potential (HP) of -70 mV. Arrows on the WT records denote the K_v current components, $I_{to,f}$, $I_{K,slow}$ and I_{ss} , previously separated and characterized in detail in WT mouse LV (and RV) cells [17,18]. The amplitudes of these currents in individual cells were determined from double exponential fits to the decay phases of the currents. In this and in subsequent **Figures**, the K_v currents in each cell were normalized to the whole-cell membrane capacitance, and current densities are plotted. (B) Mean $\pm$ SEM peak K_v current and $I_{K,slow}$ densities (at $+40$ mV) in MHC-PPAR α ($n = 25$) and WT ($n = 19$) LV myocytes are not significantly different. Mean $\pm$ SEM $I_{to,f}$ current densities, however, are significantly ($*P < 0.01$) lower in MHC-PPAR α , than WT, LV myocytes, and mean $\pm$ SEM I_{ss} densities are significantly ($**P < 0.001$) higher in MHC-PPAR α LV myocytes (see also Table 1).

Figure 2. Action potential waveforms in WT and MHC-PPAR α ventricular myocytes are indistinguishable

(A) Representative action potential waveforms, recorded from isolated WT and MHC-PPAR α LV myocytes as described in Materials and Methods, are illustrated. (B) Analyses of the resting and active membrane properties of MHC-PPAR α and WT LV myocytes revealed no significant differences in mean $\pm$ SEM resting potentials (RMP), action potential amplitudes (APA) or action potential durations (APD) at 20% (APD₂₀), 50% (APD₅₀) or 90% (APD₉₀) repolarization in WT (n = 10) and MHC-PPAR α (n = 18) LV myocytes.

Figure 3. Peak outward Kv current and $I_{to,f}$ densities are higher in PPAR α -/-, than in WT, ventricular myocytes

(A) Kv currents were recorded as described in the legend to Figure 1 from LV myocytes isolated from (5–6 week old) PPAR α -/- and WT Sv129 animals; current densities are plotted. (B) Mean $\pm$ SEM peak and $I_{to,f}$ densities (at +40mV) are significantly ($*P < 0.01$) higher in PPAR α -/- ($n = 36$), than in WT ($n = 29$), LV myocytes, whereas $I_{K,slow}$ and I_{ss} densities in PPAR α -/- and WT LV myocytes are not significantly different. Similar results were obtained in experiments on PPAR α -/- and WT RV cells (see text and Table 1).

Figure 4. Reduced Expression of *KCND2* (Kv4.2) in MHC-PPARα ventricles

K⁺ channel subunit (transcript) expression levels were examined using SYBR Green quantitative RT-PCR; primers are provided in the Materials and Methods section. Mean ± SEM subunit expression levels in 5–6 week WT (n = 6) and MHC-PPARα (n = 6) ventricles are displayed. These analyses revealed a significant (** *P* < 0.001) reduction in *KCND2* expression in MHC-PPARα ventricles, whereas the expression levels of the other I_{to,f} channel subunits, as well as of several additional K⁺ channel subunits examined, are similar in WT and MHC-PPARα ventricles. There were also no significant differences in the expression levels of the hypertrophy markers, ANF and βMHC [24] in WT and MHC-PPARα ventricles, consistent with the absence of any detectable left ventricular hypertrophy in young (5–6 week) MHC-PPARα animals [11].

Figure 5. Expression levels of the Kv4.2 and KChIP2 proteins, as well as Cx43, are markedly reduced in MHC-PPAR α ventricles
 (A–G) Representative Western blots of fractionated (5–6 week old) adult WT (n = 3) and MHC-PPAR α (n = 4) mouse ventricular proteins probed with a polyclonal anti-Kv4.2 (A), a monoclonal anti-KChIP2 (B), polyclonal anti-TASK1 (C), anti-TASK2 (D), anti-Cx43 (E), anti-Cx45 (F) antibodies or with a monoclonal anti-transferrin receptor (G) antibody. The arrows denote the protein bands corresponding to the proteins targeted by the antibodies, and the bands that were scanned for quantification of protein expression levels. (H) Films from Western blots completed on samples from (5–6 week) MHC-PPAR α (n = 10) and WT (n = 10) ventricles were scanned, and the densities of the Kv4.2, KChIP2, TASK1, TASK2, Cx43, and Cx45, as well as of the transferrin receptor, bands were measured. The densities of the bands in the MHC-PPAR α samples were normalized to the densities of the corresponding bands in the WT samples. As is evident, Kv4.2 and KChIP2 expression were significantly (* $P < 0.01$) reduced in MHC-PPAR α , compared to WT, ventricles, whereas there are no significant differences in TASK1 or TASK2 expression in MHC-PPAR α and WT ventricles. In addition,

Cx43 protein, but not Cx45 protein, expression is markedly reduced in MHC-PPAR α , compared with WT, ventricles.

Figure 6. The properties of I_{ss} in (5–6 week) WT and MHC-PPAR α ventricular myocytes are distinct

Whole-cell Kv currents were evoked in response to 10 s depolarizations to test potentials between -50 and $+5$ mV presented in 20 mV increments at 20 s intervals, and I_{ss} amplitudes were measured as the currents remaining at the end of the 10 s depolarizations. (A) Mean $\pm$ SEM I_{ss} densities are plotted as a function of test potential and, as is evident, the current-voltage relations for I_{ss} in WT ($n = 19$) and MHC-PPAR α ($n = 25$) LV myocytes are similar. (B) The current-voltage relation for the difference current, i.e., the “up-regulated” I_{ss} , in MHC-PPAR α myocytes, determined by subtraction of the mean I_{ss} currents in WT and MHC-PPAR α myocytes at each voltage, however, is distinct and is linear at voltages positive to -30 mV. (C) Mean $\pm$ SEM percent change in I_{ss} density in WT and MHC-PPAR α LV myocytes measured following exposure to various K₂P channel blockers. The fractional block of I_{ss} by gadolinium (Gd³⁺; 100 μ M; $n = 12$), zinc (Zn²⁺; 200 μ M; $n = 10$), anandamide (100 μ M; $n = 10$) and chloroform (CHCl₃; 1 mM; $n = 7$) is significantly higher (** $P < 0.001$) in MHC-PPAR α , than in WT, LV myocytes.

Figure 7. Age-related remodeling of Kv currents in MHC-PPAR α LV myocytes

(A) Representative Kv currents were recorded (as described in the legend to Figure 1) from LV myocytes isolated from 12–14 week WT and MHC-PPAR α animals. As is evident, peak Kv current amplitudes/densities in LV cells from 12–14 week MHC-PPAR α animals at all test potentials are lower than those recorded from 12–14 week WT LV cells. (B) Analyses of the current waveforms revealed that mean $\pm$ SEM I_{peak} , $I_{to,f}$ and $I_{K,slow}$ densities (at +40 mV) are significantly (** $P < 0.001$) lower in LV myocytes isolated from 12–14 week MHC-PPAR α ($n = 18$) mice, compared with cells from 12–14 week WT ($n = 37$) animals. In contrast to these observations, and similar to the findings in LV (and RV) cells from young (5–6 week) MHC-PPAR α animals, mean $\pm$ SEM I_{ss} densities in LV myocytes from 12–14 week MHC-PPAR α animals are significantly (** $P < 0.001$) higher than in WT LV myocytes. In addition, I_{ss} densities in LV myocytes from 5–6 week (Figure 1, Table 1) and 12–14 week (Table 3) MHC-PPAR α animals are not significantly different.

K⁺ Current Remodeling in Ventricular Myocytes from 5–6 week MHC-PPAR α and PPAR α -/- Mice¹

Table 1

Cells		I _{peak} ²	I _{to,f} ²	I _{K,slow} ²	I _{ss} ²	I _{K1} ³
WT (C57BL/6) LV	τ_{decay} , ms Density, pA/pF n	— 69.0 $\pm$ 3.6 19	81 $\pm$ 3 39.0 $\pm$ 2.0 19	1016 $\pm$ 36 21.9 $\pm$ 2.0 19	— 5.3 $\pm$ 0.2 19	— -11.5 $\pm$ 0.7 19
MHC-PPAR α LV	τ_{decay} , ms Density, pA/pF n	— 62.9 $\pm$ 3.5 25	90 $\pm$ 3 29.1 $\pm$ 2.3 [*] 25	1068 $\pm$ 40 21.8 $\pm$ 1.2 25	— ^{**} 9.8 $\pm$ 0.4 ^{**} 25	— -15.9 $\pm$ 0.6 ^{**} 25
WT (C57BL/6) RV	τ_{decay} , ms Density, pA/pF n	— 84.7 $\pm$ 4.1 21	85 $\pm$ 4 44.5 $\pm$ 2.1 21	927 $\pm$ 21 30.0 $\pm$ 2.2 21	— 5.8 $\pm$ 0.3 21	— -13.8 $\pm$ 0.8 21
MHC-PPAR α RV	τ_{decay} , ms Density, pA/pF n	— 79.4 $\pm$ 4.7 19	92 $\pm$ 6 36.4 $\pm$ 2.8 [*] 19	948 $\pm$ 32 29.4 $\pm$ 2.7 19	— ^{**} 10.2 $\pm$ 0.6 ^{**} 19	— -19.3 $\pm$ 0.8 ^{**} 19
WT (Sv129) LV	τ_{decay} , ms Density, pA/pF n	— 54.7 $\pm$ 3.0 29	69 $\pm$ 3 33.4 $\pm$ 1.8 29	1179 $\pm$ 50 14.9 $\pm$ 0.8 29	— 4.3 $\pm$ 0.1 29	— -10.1 $\pm$ 0.6 28
PPAR α -/- LV	τ_{decay} , ms Density, pA/pF n	— 65.5 $\pm$ 2.9 [*] 36	66 $\pm$ 3 43.9 $\pm$ 1.9 [*] 36	1103 $\pm$ 26 15.3 $\pm$ 0.9 36	— 4.9 $\pm$ 0.2 36	— -9.6 $\pm$ 0.5 17
WT (Sv129) RV	τ_{decay} , ms Density, pA/pF n	— 70.5 $\pm$ 3.7 28	61 $\pm$ 1 47.9 $\pm$ 3.1 28	1156 $\pm$ 32 15.8 $\pm$ 0.8 28	— 5.3 $\pm$ 0.2 28	— -11.7 $\pm$ 0.6 20
PPAR α -/- RV	τ_{decay} , ms Density, pA/pF n	— 86.4 $\pm$ 4.5 [*] 25	67 $\pm$ 3 56.2 $\pm$ 3.3 [*] 25	1020 $\pm$ 36 23.1 $\pm$ 1.8 [*] 25	— 6.3 $\pm$ 0.3 25	— -12.7 $\pm$ 0.8 18

¹ All values are means $\pm$ SEM; n = number of cells

² K_v current densities were determined at +40 mV (HP = -70 mV)

³ I_{K1} densities were determined at -110 mV (HP = -70 mV)

^{*} I_{to,f} densities are significantly ($P < 0.01$) lower in MHC-PPAR α , than in WT, LV and RV cells

^{**} I_{ss} and I_{K1} densities are significantly ($P < 0.001$) higher in MHC-PPAR α , than in WT, LV and RV cells.

^{*} Peak K_v and I_{to,f} densities are significantly ($P < 0.01$) higher in PPAR α -/-, than in WT, LV and RV cells.

Table 2
ECG Parameters in 5–6 week old WT, MHC-PPAR α and PPAR $\alpha^{-/-}$ Mice¹

Cells	Heart Rate (bpm)	PR (ms)	QRS (ms)	QT (ms)	QT _c (ms) ²
WT n = 17	441 ± 15	40.5 ± 1.3	9.5 ± 0.3	55.3 ± 0.9	47.5 ± 0.5
MHC-PPAR α n = 12	351 ± 11**	39.2 ± 1.7	15.7 ± 1.5**	62.1 ± 1.8**	47.8 ± 1.4
PPAR $\alpha^{-/-}$ n = 8	435 ± 18	42.3 ± 1.5	9.8 ± 0.3	56.5 ± 2.6	47.9 ± 1.7

¹ All values are means ± SEM; n = number of animals
² QT_c = corrected QT intervals calculated as QT_c = (QT/(RR/100))^{1/2}
** Values are significantly different (*P* < 0.001) from those measured in WT animals.

Table 3
Repolarizing K⁺ Currents in Ventricular Myocytes from 12–14 week WT and MHC-PPAR α Mice¹

Cells	C _m (pF) ²	τ_{decay} , ms	Density, pA/pF	I _{peak} ³	I _{tot,f} ³	I _{K,slow} ³	I _{ss} ³	I _{K1} ⁴
WT LV	157 ± 6	τ_{decay} , ms		—	95 ± 5	942 ± 24	—	—
n	37	Density, pA/pF	70.9 ± 3.6	37	37.9 ± 2.8	24.5 ± 1.2	5.0 ± 0.1	—10.9 ± 0.4
MHC-PPAR α LV	168 ± 9	τ_{decay} , ms	—	—	114 ± 18	1244 ± 103	—	34
n	18	Density, pA/pF	50.5 ± 5.4	18	21.4 ± 2.7**	16.7 ± 2.6**	11.6 ± 1.1**	—18.6 ± 1.1**
WT RV	168 ± 6	τ_{decay} , ms	—	—	95 ± 6	910 ± 27	—	—
n	26	Density, pA/pF	78.2 ± 3.3	26	37.8 ± 1.9	29.7 ± 1.8	5.8 ± 0.2	—14.3 ± 0.9
MHC-PPAR α RV	155 ± 12	τ_{decay} , ms	—	—	86 ± 13	963 ± 76	—	26
n	11	Density, pA/pF	81.9 ± 8.7	11	36.4 ± 4.4	24.8 ± 5.1	16.8 ± 1.9**	—22.0 ± 1.5**

¹ All values are means ± SEM; n = number of cells.

² C_m values were determined from integration of the capacitive transients evoked during ± 10mV voltage steps from the HP (−70 mV).

³ K_v current densities were determined at +40 mV (HP = −70mV).

⁴ I_{K1} densities were determined at −110 mV (HP = −70mV).

** Peak K_v current, I_{tot,f} and I_{K,slow} densities are significantly (*P* < 0.001) lower in MHC-PPAR α than in WT, LV cells from 12–14 week old animals.

*** Similar to the results obtained in RV and LV cells from 5–6 week old animals (see Table 1), I_{ss} and I_{K1} densities are significantly (*P* < 0.001) higher in LV and RV myocytes from 12–14 week MHC-PPAR α , compared with RV and LV cells from 12–14 week WT, animals.