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Deleting the accessory subunit KChIP2 results in loss of $I_{to,f}$ and increased $I_{K,slow}$ that maintains normal action potential configuration

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Abstract

Background—Four voltage-gated potassium currents: $I_{to,f}$ ($K_V4.2$), $I_{to,s}$ ($K_V1.4$), $I_{K,slow}$ ($K_V1.5 + K_V2.1$), and I_{SS} (TASK1) govern murine ventricular repolarization. Although the accessory subunit, KChIP2, influences $I_{to,f}$ expression, in preliminary experiments we found that action potential duration (APD) is maintained in KChIP2 knockout mice.

Objective—We tested the role of KChIP2 in regulating APD and studied the underlying ionic currents.

Methods—We used microelectrode techniques, whole-cell patch clamp studies, and real-time PCR amplification to characterize ventricular repolarization and its determinants in WT and KChIP2^{-/-} mice.

Results—Despite comparable baseline action potentials, APD was more markedly prolonged by 4-aminopyridine (4-AP) in KChIP2^{-/-} preparations. Peak K^+ current densities were similar in WT and KChIP2^{-/-} cells (mean±sem I_p : 28.3±2 (n=27) vs. 29.2±2 pA/pF (n=24), respectively; $P>0.05$). Heteropodatoxin-2 (HpTx-2, 1 μ M) had no effect on current amplitude in KChIP2^{-/-} myocytes. The current fractions sensitive to 4-AP (50 μ M and 1 mM) were larger in KChIP2^{-/-} than WT ($P<0.05$). Real-time PCR demonstrated absence of KChIP2 and increased $K_V1.5$ expression in KChIP2^{-/-} ventricular myocardium.

Conclusion—KChIP2 deficiency eliminated HpTx-2-sensitive $I_{to,f}$, but had little impact on total APD, secondary to upregulation of 4-AP-sensitive $I_{K,slow}$ in association with increased $K_V1.5$ expression. There is increased sensitivity to 4-AP-mediated APD prolongation in KChIP2^{-/-}. Thus, KChIP2 appears important for murine repolarization in circumstances of reduced repolarization reserve.

Keywords

Potassium currents; action potentials; electrophysiology; animal models; repolarization

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Introduction

Voltage-gated potassium (K_V) currents are important determinants of mammalian myocardial repolarization that are differently regulated in a variety of cardiovascular pathologies.¹⁻⁴ In mice, at least 4 pharmacologically, molecularly and kinetically distinct K_V currents have been identified^{5, 6}: $I_{to,f}$ (encoded by $K_V4.2$ and $K_V4.3$), $I_{to,s}$ ($K_V1.4$), $I_{K,slow}$ ($K_V1.5$ ⁸ and $K_V2.1$ ⁹), and I_{SS} (possibly involving the K_{2P} channel TASK1¹⁰). In contrast to larger animals, $K_V4.3$ is not required for functional $I_{to,f}$ in the mouse.¹¹

The K_V gene family encodes pore-forming α subunits, four of which comprise the functional channel. A number of accessory subunits contribute to the physiologically native current.¹² Of the K_V channel-interacting proteins (KChIPs) which primarily bind to the cytoplasmic N-terminal domain of the K_V4 α subunits^{13, 14}, only KChIP2 is expressed in heart.¹⁵⁻¹⁷ In mammalian cell lines, KChIP2 co-expression increases K_V4 -encoded current density, slows inactivation, and accelerates recovery from inactivation,^{15, 16} and also modulates $K_V1.5$ expression.¹⁸

In human subjects, heart failure is associated with decreased I_{to} ¹⁹, explained by decreased $K_V4.3$ and KChIP2 mRNA and protein levels.^{20, 21} A mutation in the pore region²² or deletion of the pore-forming segment²³ of $K_V4.2$ produce dominant-negative subunits that reduce $I_{to,f}$ and prolong action potential duration (APD) of murine myocytes. The pore deletion is associated with ventricular hypertrophy that progresses to heart failure, whereas the pore mutation does not lead to functional or structural dysfunction.^{22, 23} Deleting the entire $K_V4.2$ gene completely abolishes $I_{to,f}$; however neither APD prolongation nor hypertrophy is observed.²⁴

Although KChIP2 knockout reportedly leads to loss of I_{to} in murine cardiomyocytes²⁵, our preliminary action potential recordings from intact ventricles were comparable to those in wild-type (WT) mice. Therefore, we designed the present studies to explore the K_V -subtype specific consequences of KChIP2 deficiency. Our results demonstrate that while $I_{to,f}$ is eliminated, total K_V -current density remains unaltered. This is secondary to an upregulated 4-aminopyridine (4-AP) sensitive current, which translates to increased sensitivity to 4-AP-mediated APD prolongation.

Methods

Hearts were excised from anesthetized (0.1 mg/g ketamine, 0.01 mg/g xylazine, IP), adult (10-12 weeks), male C57BL6 WT control and KChIP2^{-/-} mice. Genotyping was performed using DNA isolated from tail samples (Allele Biotech, CA) followed by PCR amplification of KChIP2 and neomycin. All experiments were approved by the Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health).

Transmembrane action-potential recordings

Multicellular left- (LV) or right- (RV) ventricular free wall preparations were superfused with oxygenated Tyrode's solution containing (mM): NaCl 131; NaHCO₃ 18; KCl 4; CaCl₂ 1.8; MgCl₂ 0.5; NaH₂PO₄ 1.8; dextrose 5.5 (pH 7.4; T 37°C). High resistance (>30 MΩ) microelectrodes filled with 3M KCl were used to record (sub)epicardial action potentials from preparations paced at 500 ms cycle length via bipolar surface electrodes. After 2h equilibration, we recorded action potentials from 9-12 sites per preparation. Recordings were repeated 30 min after adding 1 mM 4-AP to the superfusate.

Patch-clamp recordings

Myocytes were disaggregated from the entire LV free wall by enzymatic dissociation and mechanical dispersion. Cells were maintained in enzyme-free solution at room temperature and used within 6h of isolation. Recording pipettes (1–2 M Ω) contained (mM): KCl 135; EGTA 10; HEPES 10; and glucose 5 (pH 7.2). The superfusate contained (mM): NaCl 136; KCl 4; MgCl₂ 2; CaCl₂ 1; CoCl₂ 5; tetrodotoxin (TTX; Sigma, St. Louis, MO) 0.01, HEPES 10; glucose 10 (pH 7.4; T 24°C). Cell capacitances were 184 $\pm$ 10 pF (WT) versus 172 $\pm$ 9 pF (KChIP2^{-/-}; $P>0.05$). Series resistance was compensated electronically by 80–90%; time-constants of the compensated capacitance decay were comparable in WT and KChIP2^{-/-} myocytes (393 $\pm$ 26 versus 412 $\pm$ 22 μ s, respectively; $P>0.05$). Leak currents were <100 pA and were not corrected. 4-AP (50 μ M and 1 mM⁵; Sigma) and heteropoda toxin 2 (HpTx-2; 1 μ M^{5, 26}; Sigma) were dissolved in water and added to the superfusate immediately before use.

Quantitative real-time PCR analysis

First strand cDNA was synthesized from 1 μ g total mRNA isolated from WT and KChIP2^{-/-} hearts. K_V1.4, K_V1.5, K_V2.1, K_V4.2, K_V4.3, KChIP2, and Cyclophilin A expression was determined by quantitative real-time PCR (Roche, Basel, Switzerland). Amplicons were controlled by gel electrophoresis and sequencing. cDNA synthesis without reverse transcriptase served as negative control and showed no amplification. Results are presented as KChIP2^{-/-} normalized to WT levels. Samples were tested in duplicate.

Data analysis

Peak outward currents (I_p) were defined as maximal outward K⁺ current from 0 pA; I_{K1} amplitudes were measured at peak inward current and at the end of the 400 ms voltage step. The decay phase of the total K_V current elicited by 4.5-s depolarization in LV free wall myocytes can be described by the sum of 2 exponential components, where the fast and slow τ are attributed to $I_{to,f}$ and $I_{K,slow}$, respectively.^{6, 24} $I_{to,s}$ is found exclusively in cells from the interventricular septum. The steady-state current, I_{ss} , does not inactivate and is described by a time-independent, constant current.⁷ Only fits with a correlation coefficient $\geq 98\%$ were used. For each cell, current amplitudes are normalized to the whole-cell membrane capacitance, and current densities reported.

Data are presented as mean $\pm$ SEM. Student's t test (when comparing 2 data sets) or 2-way ANOVA, followed by Bonferroni's test, when appropriate, were used to test differences statistically. $P<0.05$ was considered significant.

Results

Exaggerated APD prolongation by 4-AP in KChIP2^{-/-} ventricles

Maximum diastolic potential, action potential amplitude and V_{max} were comparable between WT and KChIP2^{-/-} groups (Table). WT and KChIP2^{-/-} APD were similar at control and prolonged significantly after exposure to 1 mM 4-AP (Table, Figure 1). However, 4-AP induced more marked APD prolongation in KChIP2^{-/-} LV and RV preparations. In RV, 4-AP effects were greater in KChIP2^{-/-} than WT ($P<0.05$; Table). Thus, the altered repolarizing currents in KChIP2^{-/-} mice manifest as APD changes only in the presence of 4-AP; baseline APDs are equivalent to WT. This prompted us to characterize the voltage-gated potassium currents in disaggregated myocytes from WT and KChIP2^{-/-} mice.

Comparable peak K_V currents in WT and KChIP2^{-/-} ventricular myocytes

Figure 2A shows representative K⁺ currents recorded from a WT and a KChIP2^{-/-} myocyte. Mean peak current (I_p) densities at +40 mV are 28.3 $\pm$ 2.0 pA/pF in WT cells (n=27) versus

29.2±1.9 pA/pF in KChIP2^{-/-} cells (n=24; $P>0.05$). No differences in I_P density were seen using 4.5-s depolarizing steps between -40 and +50 mV ($V_H=-70$ mV; Figure 2B). Activation kinetics, measured as the time from depolarization to peak current, were significantly slower in KChIP2^{-/-} compared to WT, particularly at steps to positive voltages (Figure 2C).

Significant differences were found in the double-exponential fit to the 4.5-s decay phase of the K_V currents. At $V_{step}=+40$ mV, the fast τ were 198±11 versus 226±19 ms in WT and KChIP2^{-/-} myocytes, respectively ($P<0.05$). The slow τ were 1486±57 versus 1248±69 ms in WT and KChIP2^{-/-} myocytes, respectively ($P<0.05$). The peak amplitudes of the fast component (9±2 versus 12±2 pA/pF, $P>0.05$), slow component (12±1 versus 12±1 pA/pF, $P>0.05$), and the constant component (6±1 versus 7±1 pA/pF, $P>0.05$), were all comparable between WT and KChIP2^{-/-} myocytes, respectively. When 400-ms depolarizations were used, a fast inactivating component was clearly visible in WT cells but absent in KChIP2^{-/-} myocytes (Figure 2D). This fast component had a time constant of inactivation of 38±2 ms, which is comparable to the previously reported τ for $I_{to,f}$.⁶

We measured peak and steady-state I_{K1} to address whether other K^+ currents were affected by KChIP2^{-/-} (Figure 3A). No measurable differences in the inwardly rectifying K^+ current were observed in KChIP2^{-/-} (n=7-10; Figure 3B).

No HpTx-2-sensitive current and larger 4-AP-sensitive currents in myocytes from KChIP2^{-/-} mice

To evaluate the contribution of KChIP2 we used HpTx-2 (1 μ M), which selectively blocks $I_{to,f}$ without affecting other murine outward K^+ currents.²⁶ Figure 4A shows native K_V currents in representative WT and KChIP2^{-/-} myocytes. Addition of HpTx-2 abolished the fast-inactivating component of the K_V -current decay in the WT cell and reduced I_P (Figure 4B). HpTx-2 blocked 11±2% of the I_P of the native K_V current in WT myocytes (n=13; Figure 4C), but reduced the current by only 3±2% in KChIP2^{-/-} myocytes (n=9; $P<0.05$ versus WT), suggesting KChIP2^{-/-} mice lack the HpTx-2 sensitive $I_{to,f}$.

Since total K_V currents are comparable in WT and KChIP2^{-/-} mice, but $I_{to,f}$ is missing in KChIP2^{-/-} myocytes, it is possible that a non- K_V4 current is increased in KChIP2^{-/-} mice. Therefore, we used a pharmacological approach to dissect the remaining currents in WT and KChIP2^{-/-} myocytes. 4-AP ≥ 5 mM abolishes all outward K^+ currents in mice. At lower concentrations ($IC_{50} \approx 50$ μ M), 4-AP most potently blocks $I_{K,slow}$, whereas $I_{to,f}$ is blocked at higher concentrations ($IC_{50} \approx 500$ μ M). I_{SS} is blocked only in the mM range.⁷

4-AP concentration-dependently decreased peak-current densities in WT and KChIP2^{-/-} myocytes (Figure 5). Figure 5A illustrates total K_V current before and after adding 50 μ M 4-AP to the superfusate in WT and KChIP2^{-/-} myocytes. The 4-AP-sensitive current was obtained by digitally subtracting this current from the original K_V current (Figure 5B). Peak 4-AP-sensitive current amplitude was significantly larger in KChIP2^{-/-} myocytes than in WT myocytes at both 4-AP concentrations (Figure 5C). 4-AP-insensitive currents showed clear time-dependent inactivation in all WT myocytes (Figure 5A, left), whereas the 4-AP-insensitive current in KChIP2^{-/-} myocytes showed very little inactivation over the depolarizing step (Figure 5A, right). When I_P of 4-AP-insensitive (1 mM) currents were measured within the first 100 ms of the voltage step and compared to the mean current amplitude in the last 100 ms of the voltage step, 82±4% (n=12) of peak current remained in KChIP2^{-/-} cells, compared to 58±2% in the WT myocytes (n=9; $P<0.05$). This suggests the absence of time-dependent current in KChIP2^{-/-} myocytes exposed to 1 mM 4-AP.

Elevated $K_v1.5$ transcription in $KChIP2^{-/-}$ mice

The altered pharmacological responsiveness combined with an unaltered native I_p in $KChIP2^{-/-}$ myocytes suggest that currents contributing to overall repolarization may change subsequent to $KChIP2$ knockout. Therefore, we studied the molecular remodeling in $KChIP2^{-/-}$ LV, using quantitative real-time PCR with K_v -subunit specific primers to quantify transcriptional activity in WT (n=6) and $KChIP2^{-/-}$ (n=6) tissue. We found significant increases in mRNA levels for $K_v4.2$, $K_v4.3$, and $K_v1.5$ in $KChIP2^{-/-}$ ventricles versus WT (Figure 6). As expected, $KChIP2$ transcription was not detected in $KChIP2^{-/-}$ LV. $K_v2.1$ and $K_v1.4$ mRNA were comparable in WT and $KChIP2^{-/-}$ LV. Cyclophilin A levels (control) remained unaltered.

Discussion

The significant findings of this study are that myocytes from $KChIP2^{-/-}$ mice have no $I_{to,f}$, but show no change in I_p density, versus WT myocytes. An increased 4-AP-sensitive current is likely responsible for the maintained I_p in $KChIP2^{-/-}$ mice. Transmembrane action potentials recorded from intact ventricular preparations under control conditions are comparable in $KChIP2^{-/-}$ and WT; however, 4-AP-induced APD prolongation is intensified in $KChIP2^{-/-}$ preparations. Furthermore, the transcript level of the $K_v1.5$ subunit is increased in $KChIP2^{-/-}$ ventricles. In total, this is consistent with an augmented $I_{K,slow}$ in $KChIP2^{-/-}$ myocytes, maintaining normal repolarization reserve.

$KChIP2$ is responsible for maintenance of mouse ventricular $I_{to,f}$

Using 4.5s depolarizing steps, K_v current decay was described by 2 exponential components in $KChIP2^{-/-}$ and WT myocytes, with only minor differences in time constants of inactivation. A very fast component of inactivation ($\tau < 50$ ms) was present in WT myocytes within the first 100 ms of depolarization, and by using 400-ms pulses, this rapid component was amplified relative to the total current decay (compare Figure 2A and 2D). This component was blocked by HpTx-2, which had no effect in $KChIP2^{-/-}$ myocytes (Figure 4). Thus, we found no evidence that $K_v4.2$ or $K_v4.3$ produce functional $I_{to,f}$ in the absence of $KChIP2$ in the mouse, despite increased mRNA levels of these α subunits in the LV (Figure 6).

The unaltered I_p and APD in the absence of K_v antagonists in $KChIP2^{-/-}$ mice suggest upregulation of a secondary repolarizing K^+ current that compensates for the absent $I_{to,f}$. Interestingly, 4-AP had a larger effect on K_v current amplitude in $KChIP2^{-/-}$ myocytes, translating to exaggerated APD prolongation upon 4-AP-treatment. Previous studies suggest that $KChIP2$ associates with the $K_v1.5$ α subunit in myocytes and that $KChIP2$ co-expression decreases $K_v1.5$ -encoded K^+ current in cell lines, suggesting a multifunctional role of $KChIP2$ in regulating murine repolarizing currents.¹⁸ Thus, by relieving $KChIP2$ -mediated suppression of $K_v1.5$ in $KChIP2^{-/-}$ mice, an increased $I_{K,slow}$ would be expected. We observe a 3-fold increase in $K_v1.5$ mRNA in $KChIP2^{-/-}$ ventricles (Figure 6) and an augmented 4-AP-sensitive $I_{K,slow}$ (Figure 5). The lack of an anti- $K_v1.5$ antibody that can detect endogenous $K_v1.5$ in cardiomyocytes reliably²⁷ precluded analysis of $K_v1.5$ protein expression. Furthermore, it is unclear whether direct relief of suppression contributes to the increased $I_{K,slow}$ in $KChIP2^{-/-}$ myocytes.

In vitro, $KChIP2$ increases K_v4 -encoded current amplitude, slows inactivation, and accelerates recovery from inactivation.^{15, 16} Hence, if a K_v4 -dependent current plays a considerable role in murine outward voltage-gated K^+ currents, corollaries of $KChIP2^{-/-}$ *in vivo* should include decreased I_p density, faster current decay, and slowed recovery of I_p from inactivation. Despite transcription of $K_v4.2$ and $K_v4.3$, we found no residual $I_{to,f}$ in $KChIP2^{-/-}$ mice. Thus, although a small, fast inactivating current is recorded in cell lines transfected with $K_v4.2$ or $K_v4.3$ in

the absence of KChIP2, no such current is found in KChIP2^{-/-} mice, suggesting an essential role for KChIP2 in translating Kv4 protein into functional current *in vivo*.

***I*_{to} remodeling in KChIP2^{-/-} mice reduces repolarization reserve**

The KChIP2^{-/-} mouse adds to the animal models and patient data in which a normal QT interval is excessively prolonged by a challenge (e.g. *I*_{Kr} block), reflecting a reduced repolarization reserve.^{29, 30} Many patients with congenital or drug-induced long-QT syndromes have normal QT intervals in the absence of provocation, referred to as genotype-phenotype discordance.^{31, 32} Furthermore, downregulation of *I*_{to} is prominent in heart failure^{1, 4}, where *I*_{Kr}-blocking drugs can induce excess QT interval prolongation.³³ Additionally, the significant cardiac plasticity in cardiac memory in dogs³⁴, includes decreased *I*_{to} and downregulated Kv4.3 and KChIP2 transcription in myocardium near ventricular pacing sites.^{35, 36} Here, *I*_{Kr} blockade causes more marked QT prolongation than in control dogs.³⁷

In the present study, removal of *I*_{to,f} from the assembly of murine repolarizing currents caused no overt reduction in total current (Figure 2), due to *I*_{K,slow} upregulation. The phenotypic consequence of this remodeling became evident only when action potentials were recorded in the presence of 4-AP (Figure 1). Thus, a reduced repolarization reserve with respect to *I*_{to} increases susceptibility to pharmacological prolongation of repolarization. Finally, the differences in action potential morphology and 4-AP response between LV and RV (Figure 1) likely reflect regional differences in repolarizing currents reported in cells isolated from the ventricles.⁵

Relationship to other studies on mouse pathologies

Kuo *et al.*²⁵ reported decreased total *I*_{to} density and significantly prolonged APD in disaggregated RV myocytes from KChIP2^{-/-} mice. In their study, *I*_{Na} and *I*_{Ca,L} were allowed to contribute to the current recordings.^{25, 38} In contrast, our study evaluated the relative contributions of the different voltage-dependent K⁺ currents to repolarization in KChIP2^{-/-} mice. This intent prompted us to use solutions containing antagonists for *I*_{Na} (TTX) and *I*_{Ca,L} (Co²⁺).⁶ Using protocols and solutions identical to those of Kuo *et al.*, we observed a smaller total outward current in KChIP2^{-/-} myocytes (data not shown). The discrepancy in APD likely reflects the difference between the uncoupled disaggregated myocytes in the previous study²⁵ and the multicellular preparations in the present study. However, our results complement the previous report of unaltered QT intervals or effective refractory periods in anesthetized, intact KChIP2^{-/-} mice.²⁵

In agreement with earlier reports on KChIP2^{-/-} mice, we observed no evidence of cardiac hypertrophy or impaired function.²⁵ This is paralleled by results from Kv4.2^{-/-} mice²⁴ and mice expressing a Kv4.2-pore mutant.²² In contrast, mice expressing a carboxy-terminal deletion of Kv4.2 display marked hypertrophy and ventricular dilatation progressing to congestive heart failure.²³ Whereas the two mutants display prolonged APD, Kv4.2^{-/-} mice have an upregulated *I*_{to,s} and increased transcription of Kv1.4.²⁴ In our experiments, we found neither *I*_{to,s} nor increased Kv1.4 mRNA in KChIP2^{-/-} myocytes. Thus, the compensatory mechanisms clearly differ between Kv4.2^{-/-} and KChIP2^{-/-} mice and the signal transduction causing this discrepant remodeling is of interest. Furthermore, the absence of functional remodeling in the two knockout mice argues against a direct link between deficient *I*_{to,f} and cardiac hypertrophy and heart failure.

Limitations

Our recordings of WT *I*_P are smaller and *I*_{to,f} are reduced versus other reports⁶, despite identical mouse strains, patch-clamp solutions, and voltage protocols. Different cell-disaggregation protocols or storage conditions between myocytes disaggregation and patch-clamp

experiments (plating on laminin-coated coverslips, stored in 5% CO₂ incubator in up to 24 h)⁶ could contribute to the differences seen between laboratories. Nevertheless, we studied WT and KChIP2^{-/-} mice under identical conditions and the differences reported are consequent to KChIP2 deficiency.

Although increased mRNA expression may not be identical to increased protein translation and –trafficking, our functional data of upregulated $I_{K,slow}$ density complements the increased $K_V1.5$ mRNA levels.

Conclusions

Loss of KChIP2 has little impact on total murine K_V current, and the genotype is reflected phenotypically only during pharmacological challenges to repolarization. $I_{to,f}$ is absent in KChIP2^{-/-} mice, whereas 4-AP-sensitive $I_{K,slow}$ is upregulated. This is associated with excess prolongation of APD in the presence of 4-AP and with increased expression of $K_V1.5$. Thus, KChIP2 and $I_{to,f}$ appear critical to action potential morphology in the mouse only in circumstances of decreased repolarization reserve.

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Figure 1. APD prolongation by 4-AP is enhanced in KChIP2^{-/-} ventricles. Representative action potentials recorded from intact WT and KChIP2^{-/-} LV and RV in control and after addition of 1 mM 4-AP.

Figure 2.

Comparison of K_V currents in WT and KChIP2^{-/-} myocytes. **A**, Representative current traces normalized to cell capacitance recorded from WT and KChIP2^{-/-} cells using 4.5-s depolarizing steps from $V_H = -70$ mV to $V_{step} = -40$ to $+50$ mV (Inset). Each step was preceded by a 25 ms depolarization to -20 mV to eliminate contamination by inward Na^+ current not completely blocked by TTX. Arrowheads indicate 0 pA. **B**, I_p densities plotted as a function of V_{test} in WT ($n=27$) and KChIP2^{-/-} ($n=24$) myocytes. **C**, Time interval from cell depolarization to peak current as a function of V_{test} . *, $P < 0.05$ versus WT. **D**, Representative current traces normalized to cell capacitance recorded from a WT and a KChIP2^{-/-} cell using 400-ms depolarizing steps.

Although peak current amplitude is comparable, the WT cell displays faster current inactivation within the first ~100 ms.

Figure 3. Similar inward rectifying K⁺ currents in WT and KChIP2^{-/-} myocytes. **A**, Representative current traces recorded from a WT and KChIP2^{-/-} myocyte. Arrowheads indicate 0 pA. Inset, voltage protocol. **B**, Peak inward I_{K1} (left) and steady state (right) I_{K1} in WT (n=7) and KChIP2^{-/-} (n=10) myocytes.

Figure 4.

No effect of HpTx-2 on K_V currents in KChIP2^{-/-} myocytes. **A**, Representative baseline K_V currents recorded using 400-ms pulses from $V_H = -70$ mV to V_{step} between -40 and +50 mV (inset) in myocytes from WT and KChIP2^{-/-} mice. Arrowheads indicate 0 pA. **B**, Currents recorded in the same myocytes after addition of 1 μ M HpTx-2 to the superfusion solution. **C**, The HpTx-2-sensitive currents obtained by digital offline subtraction of the current traces shown in A and B.

Figure 5.

Larger fraction of K_V current is sensitive to 4-AP in KChIP2^{-/-} myocytes. **A**, Current traces normalized to cell capacitance recorded using 400-ms pulses from $V_H = -70$ mV to $V_{step} = +40$ mV from a WT and a KChIP2^{-/-} cell, before and after addition of 50 μM 4-AP to the superfusate. Arrowheads indicate 0 pA. Note the prominent inactivation of the 4-AP resistant current in WT. **B**, The current fraction sensitive to 50 μM 4-AP obtained by digital offline subtraction of the current traces shown in A. **C**, 4-AP-sensitive current relative to the full I_p in WT and KChIP2^{-/-} cells. *, $P < 0.05$.

Figure 6.

Compensatory remodeling potentially includes elevated $K_{V1.5}$ transcription. Expression levels of the major ion channels underlying murine repolarization in $KChIP2^{-/-}$ (n=6) relative to WT (n=6) ventricles. Grey horizontal line indicates equal expression levels in WT and $KChIP2^{-/-}$ ventricles. $KChIP2$ was not detected in $KChIP2^{-/-}$ myocytes. *, $P < 0.05$.

Table
Action potential characteristics from intact LV and RV preparations.

LV	n	APA, mV	MDP, mV	V _{max} [*] V/s	APD ₅₀ , ms	APD ₉₀ , ms
WT	4	73±3	-69±2	110±27	11±1	75±10
WT, 4-AP	4	76±3	-69±2	107±15	20±1 [*]	84±3 [*]
KChIP2 ^{-/-}	3	70±3	-67±2	87±11	10±1	70±4
KChIP2 ^{-/-} , 4-AP	3	74±6	-67±1	93±5	21±1 [*]	84±2 [*]
RV	n	APA, mV	MDP, mV	V _{max} [*] V/s	APD ₅₀ , ms	APD ₉₀ , ms
WT	8	88±2	-77±1	215±12	4.1±0.3	31±3
WT, 4-AP	8	87±3	-73±1	196±15	8.9±0.8 [*]	41±4 [*]
KChIP2 ^{-/-}	7	87±2	-77±1	192±20	3.8±0.3	39±4
KChIP2 ^{-/-} , 4-AP	7	93±2	-74±2	160±13	13.6±0.9 ^{*†}	53±4 ^{*†}

APA, action potential amplitude; MDP, maximal diastolic potential; V_{max}, maximal upstroke velocity; APD₅₀ and APD₉₀, action potential duration to 50 and 90% repolarization. Relative increases in APD induced by 4-AP are indicated in percent. Pacing-cycle length: 500 ms.

^{*}, *P*<0.05 versus baseline.

[†], *P*<0.05 versus WT, 4-AP.