Ca/Calmodulin Kinase II Differentially Modulates Potassium Currents

Stefan Wagner, MD¹; Elena Hacker, MD¹; Eleonora Grandi, PhD²; Sarah L. Weber¹, Nataliya Dybkova, PhD¹; Samuel Sossalla, MD¹; Thomas Sowa¹, Larissa Fabritz, MD³; Paulus Kirchhof, MD³; Donald M. Bers, PhD²; Lars S. Maier, MD¹

¹Dept. Cardiology & Pneumology, Georg-August-University Göttingen, Germany
²Dept. Pharmacology University of California, Davis, USA
³Dept. Cardiology & Angiology, University-Hospital Münster, Germany

Corresponding author: Prof. Dr. med. Lars S. Maier
Heisenberg Professor
Dept. Cardiology & Pneumology
Georg-August-University Göttingen
Robert-Koch-Str. 40
37075 Göttingen
Germany
Tel.: +49-551-399481, Fax: +49-551-398941
Email: Imaier@med.uni-goettingen.de

Running Head: CaMKII regulates potassium currents

Key Words: action potentials, potassium, arrhythmia, electrophysiology, heart failure

Subject codes: [132] arrhythmias-basic studies, [148] Heart failure – basic studies, [152] Ion channels/membrane transport
Abstract

Background: Potassium currents contribute to action potential duration (APD) and arrhythmogenesis. In heart failure, Ca/calmodulin-dependent protein kinase II (CaMKII) is upregulated and can alter ion channel regulation and expression.

Methods and Results: We examine the influence of overexpressing cytoplasmic CaMKIIδC, both acutely in rabbit ventricular myocytes (24 h adenoviral gene transfer) and chronically in CaMKIIδC-transgenic mice, on transient outward potassium current (Ito), and inward rectifying current (IK1). Acute and chronic CaMKII overexpression increases Ito,slow amplitude and expression of the underlying channel protein Kv1.4. Chronic, but not acute, CaMKII overexpression, causes down-regulation of Ito,fast, as well as Kv4.2 and KChIP2, suggesting that Kv1.4 expression responds faster and oppositely to Kv4.2 upon CaMKII activation. These amplitude changes were not reversed by CaMKII inhibition, consistent with CaMKII-dependent regulation of channel expression and/or trafficking. CaMKII (acute and chronic) greatly accelerated recovery from inactivation for both Ito components, but these effects were acutely reversed by AIP (CaMKII inhibitor), suggesting that CaMKII activity directly accelerates Ito recovery. Expression levels of IK1 and Kir2.1 mRNA were downregulated by CaMKII overexpression. CaMKII acutely increased IK1, based on inhibition by AIP (in both models). CaMKII overexpression in mouse prolonged APD (consistent with reduced Ito,fast and IK1), while CaMKII overexpression in rabbit shortened APD (consistent with enhanced IK1 and Ito,slow and faster Ito recovery). Computational models allowed discrimination of contributions of different channel effects on APD.

Conclusion: CaMKII has both acute regulatory effects and chronic expression level effects on Ito and IK1 with complex consequences on APD.
**Introduction**

Heart failure (HF) is accompanied by arrhythmogenic changes related to electrical remodeling. This is associated with prolongation of action potential duration (APD) and down-regulation of transient outward K-current ($I_{to}$) and inward rectifying K-current ($I_{K1}$). $I_{K1}$ is responsible for stabilizing the diastolic membrane potential ($E_m$), such that decreased $I_{K1}$ increases the propensity for triggered arrhythmias. $I_{to}$ is important in early repolarization and influences the effects of other currents and transporters by affecting AP voltage-time trajectory. There are at least two components of $I_{to}$ generated by different K-channel isoforms, which can be distinguished according to their recovery and inactivation kinetics. The fast component ($I_{to,fast}$) recovers and inactivates with time constants ($\tau$) of <100 ms, whereas the slow component ($I_{to,slow}$) recovers with $\tau$ of hundreds of milliseconds up to several seconds and inactivates with $\tau$ of ~200 ms. Downregulation of $I_{to}$ has been described in animal models of hypertrophy and human HF, is associated with APD prolongation and predisposes to early after-depolarizations.

In HF, expression and activity of Ca/calmodulin-dependent kinase II (CaMKII) are enhanced. CaMKII is the predominant cardiac isoform and transgenic mice (TG) overexpressing cytosolic CaMKII develop HF with increased APD and are prone to ventricular arrhythmias. Recent evidence suggests that chronic inhibition of CaMKII results in APD shortening and prevents remodeling after myocardial infarction and excessive $\beta$-adrenergic stimulation. Moreover, an enhancement of $I_{to}$ and inward rectifying $I_{K1}$ after chronic CaMKII inhibition was described, whereas acute CaMKII-inhibition did not increase $I_{to}$ and $I_{K1}$.

We studied how CaMKII alters $I_{to}$ and $I_{K1}$, both acutely by adenoviral CaMKII overexpression in rabbit myocytes and chronically in CaMKII TG mice. We found that CaMKII activation exerts an acute regulatory effect on $I_{to}$ and $I_{K1}$, and that CaMKII causes opposite effects on functional expression of $I_{to,slow}$ and $I_{to,fast}$, whereas functional expression of $I_{K1}$ was only downregulated after TG CaMKII overexpression.
**Methods**

**CaMKII\(\delta\)C TG mice and overexpression of CaMKII\(\delta\)C in rabbit myocytes**

CaMKII\(\delta\)C TG mice were used at 17.6±2.3-weeks of age and compared to their age- and sex-matched wild-type (WT) littermates. Ventricular myocytes were isolated and kept in modified Tyrode solution containing (mmol/l) 137 NaCl, 5.4 KCl, 1.2 MgSO\(_4\), 1.2 Na\(_2\)HPO\(_4\), 20 HEPES, 15 glucose, 1 CaCl\(_2\) (pH 7.4). Ventricular myocytes were isolated from rabbits (1.3-2.0 kg). Transfection with CaMKII\(\delta\)C adenovirus (Ad-CaMKII\(\delta\)C) was performed and compared to \(\beta\)-galactosidase (Ad-\(\beta\)Gal) as a control at a MOI of 100. Cells were cultured for 24 h with M199 and washed prior to the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee.

An expanded Materials and Methods section can be found in the online data supplement.

**Results**

**Functional expression and inactivation kinetics of \(I_{to}\)**

To measure CaMKII\(\delta\)C-dependent regulation of \(I_{to}\), the \(E_m\)-dependence of activation and the kinetics of inactivation were analyzed (Figure 1-2). \(I_{to,fast}\) and \(I_{to,slow}\) were separated based on inactivation rate. Original traces were fit to bi-exponentials to obtain amplitudes and time constants \(\tau_{fast}\) and \(\tau_{slow}\) of inactivation. In CaMKII\(\delta\)C TG mice, total \(I_{to}\) was significantly reduced (vs. WT, Fig 1A), due to a large reduction in \(I_{to,fast}\) with a slightly smaller increase in \(I_{to,slow}\) (Fig 1C-D).

In CaMKII\(\delta\)C mice, neither the \(E_m\)-dependence of \(I_{to}\) activation (both \(I_{to}\) components), nor the inactivation kinetics for \(I_{to,fast}\) nor the steady-state current were altered (Fig 1C-E). However, \(I_{to,slow}\) inactivated more slowly in the CaMKII\(\delta\)C mice (Figure 1C, right). Neither the \(I_{to}\) amplitude effects nor the inactivation kinetic effects in CaMKII\(\delta\)C mice were reversed by CaMKII-inhibition using AIP, implying that CaMKII alters functional channel (or regulator) expression.

In rabbit myocytes, CaMKII\(\delta\)C-overexpression significantly enhanced total \(I_{to}\) (Fig 2A). This was the result of significant up-regulation of \(I_{to,slow}\) amplitude, without detectable alteration in \(I_{to,fast}\) (Fig 2C-D). No CaMKII-dependent shifts were detected in either the \(E_m\)-dependence or kinetics of inactivation. Again, the CaMKII-induced changes in total \(I_{to}\) and \(I_{to,slow}\) amplitude were
not reversible using AIP, suggesting that functional upregulation of $I_{to,slow}$ may occur after only 24 hr of CaMKII$_{\delta C}$ adenoviral infection.

**$I_{to}$ recovery from inactivation**

$I_{to}$ recovery from inactivation was assessed using a two pulse protocol (Fig 3A). Compared to WT and $\beta$-gal control myocytes, $I_{to}$ recovery from inactivation was significantly faster in CaMKII$_{\delta C}$-overexpressing mouse (Fig 3B-C) and rabbit (Fig 4A) myocytes. This accelerated recovery was completely reversed by AIP (Fig 4B). Recovery was bi-exponential and CaMKII accelerated both fast ($k_{fast}$) and slow ($k_{slow}$) recovery from inactivation, an effect reversed by AIP (Fig 4B, Table 1). Thus, CaMKII appears to acutely speed recovery of $I_{to,fast}$ and $I_{to,slow}$, and this would increase $I_{to}$ availability at high heart rates.

Since $I_{to,fast}$ typically inactivates and recovers faster than $I_{to,slow}$, we compared the amplitudes of $I_{to}$ recovery. CaMKII significantly increased the $I_{to,slow}$ proportion ($A_{slow}/(A_{slow}+A_{fast})$) and $I_{to,slow}$ density in both rabbit and mouse, but this effect was not reversed by AIP (Fig 4C, Table 1). This may reflect a CaMKII-dependent increase in functional expression of $I_{to,slow}$. In contrast, $I_{to,fast}$ density was not altered by CaMKII overexpression in rabbit, suggesting unaltered functional expression of $I_{to,fast}$. Conversely, CaMKII$_{\delta C}$ mice exhibit decreased $I_{to,fast}$ (Fig 4C, Table 1) which was not reversible by AIP. These conclusions match those from the inactivation kinetics (Figs 1-2) and strengthen the conclusions regarding $I_{to}$ assignment to $I_{to,fast}$ and $I_{to,slow}$.

**K-channel expression and co-localization of CaMKII with Kv1.4, 4.2, and 4.3**

To clarify whether there is a combination of altered channel expression changes (insensitive to AIP) as well as acute CaMKII modulation (reversed by AIP), we assessed mRNA and protein expression levels of the channel forming subunits thought to underlie $I_{to,slow}$ (Kv1.4) and $I_{to,fast}$ (Kv4.2/4.3) and the $\beta$-subunit KChIP2 that associates with Kv4.2/Kv4.3.

Kv1.4 expression was significantly enhanced by CaMKII$_{\delta C}$ expression in mice and rabbits overexpressing CaMKII$_{\delta C}$ (Fig 5A-B). This agrees with the observed increase of $I_{to,slow}$ that is not acutely blockable by AIP. Notably, mRNA measurements (Suppl. Fig. 1A+B) roughly paralleled this data, consistent with CaMKII-dependent transcriptional regulation.

In contrast to the enhanced Kv1.4, expression, Kv4.2 was significantly down-regulated in TG mice vs. WT at both protein and mRNA levels (Fig 5A and Suppl. Fig. 1), while Kv4.3
expression was unaltered. The reduction of Kv4.2 protein expression was relatively small compared to the greatly reduced $I_{\text{to, fast}}$ density. However, mRNA levels of KChIP2 were also reduced to about 50% in TG vs. WT (Suppl. Fig. 1A). Thus both Kv4.2 and KChIP2 decreases may contribute to the net reduction of $I_{\text{to, fast}}$ density. This reduced expression of Kv4.2/4.3 agrees with previously published data from failing myocardium. Reduced $I_{\text{to, fast}}$ generated via Kv4.2/4.3 may be a general feature of cardiac remodeling in HF, and this might be partly caused by CaMKII.

In rabbit myocytes, CaMKIIδC failed to alter $I_{\text{to, fast}}$ (Fig 2D&4C) and there was likewise no significant change in Kv4.3 protein or Kv4.2 or Kv4.3 mRNA expression (Fig 5B and Suppl. Fig. 1B). There was an increase in KChIP2 mRNA levels (Suppl. Fig. 1B), but that may be unrelated to the unaltered $I_{\text{to, fast}}$ amplitude.

Subcellular immunolocalization of CaMKII and K-channels (Fig 5C-D) show Kv1.4, Kv4.3 and CaMKII in rabbit myocytes. All proteins show a striated pattern, consistent with a co-localization in the transverse tubules.

**Inward rectifying current $I_{k1}$**

CaMKII effects on $I_{k1}$ were assessed using 500 ms pulses to varying $E_m$ (I-V curves in Figure 6A). We compared $I_{k1}$ (which reversed near -80 mV) using the outward current amplitudes at negative $E_m$. Measuring $I_{k1}$ in the presence of AIP showed 30-40% $I_{k1}$ down-regulation upon both acute and chronic CaMKIIδC overexpression (▲ vs. △ in Fig 6). In accordance with this, Kir2.1 gene expression was downregulated by ~40% in TG mice, and the mean value was lower in CaMKIIδC rabbit myocytes (but not significantly; Suppl. Fig. 1). Thus, CaMKII downregulates $I_{k1}$ expression within 24 h.

In all cases, the measured $I_{k1}$ was reduced by AIP (squares vs. triangles, Fig 6A), from which we infer that there may be some basal level of CaMKII-dependent $I_{k1}$ activation. In the TG mouse the AIP-sensitive component of $I_{k1}$ was reduced, implying unaltered basal CaMKII-dependent regulation. However, in the rabbit, CaMKIIδC overexpression increased the AIP-sensitive $I_{k1}$ by >100% despite a lower baseline. This raises the intriguing possibility that short-term enhancement of CaMKII can activate $I_{k1}$. These changes were seen both in the inward
component as well as the small but physiologically relevant outward component of $I_{K1}$ (not shown).

**APD and computational modeling**

Figure 7A-B show that in CaMKII$_{δC}$ TG mice, APD was significantly prolonged over a broad range of frequencies, and that AIP did not reverse the effect. This result is consistent with the reductions in $I_{to,fast}$, total $I_{to}$ and $I_{K1}$, which would all be expected to prolong APD. The lack of effect of AIP is consistent with the APD prolongation being due to the reduced expression of $K_{v}4.2$, KChIP2 and Kir2.1, rather than the acute CaMKII-dependent acceleration of $I_{to}$ recovery.

In striking contrast, rabbit APD was significantly shortened by acute CaMKII$_{δC}$ overexpression (vs. β-Gal), and this effect was largely reversed by AIP (Fig 7A&C). The APD shortening could be partly due to the CaMKII-induced enhancement of total $I_{to}$ and $I_{to,slow}$ amplitude (Figs 2C&4C) and higher $K_{v}1.4$ expression (Fig 5B), but that should not be AIP-sensitive. A more likely dominant explanation could be the CaMKII-induced acceleration of $I_{to}$ recovery from inactivation (Fig 4B), which would increase $I_{to}$ density during the AP. This would be especially the case for $I_{to,slow}$ which takes several seconds to recover in the absence of CaMKII but where CaMKII doubles the rate of recovery (Fig 4A-B). A third explanation is the CaMKII-dependent increase of $I_{K1}$ density (Fig 6A), although this would come in to play late in the AP. CaMKII may also acutely modulate other ion channels.$^{14,26}$

Because of the multiple currents during the AP, computational modeling can help identify the relative contributions of various CaMKII-sensitive currents. We made use of our rabbit ventricular AP model$^{27}$ and a mouse ventricular AP model$^{28}$ with some modifications to include CaMKII-dependent alterations on $I_{Ca}$ and $I_{Na}$$^{13,14,18}$ and the novel $I_{to}$ and $I_{K1}$ data (see online supplement). Figure 7A (insets) shows the calculated APs and Suppl. Table 2 shows the predicted APD60 and 80. Over the full range of stimulation frequencies explored APD60 and 80 are increased in the TG-CaMKII$_{δC}$ myocytes vs. WT (Suppl. Table 2). In the TG mouse, CaMKII effects on $I_{Na}$ and $I_{Ca}$ do not significantly impact the APD, whereas reduced $I_{to,fast}$ and $I_{K1}$ conspire to prolong the AP. AP prolongation is not reversed at fast rates where TG-CaMKII$_{δC}$ $I_{to}$ recovery is faster.
Using our rabbit ventricular myocyte computer model, we showed that the increased rate of $I_{to}$ recovery and enhancement of $I_{to,slow}$ are essential for the observed APD shortening. In the present study the data on $I_{K1}$ was added to the model, but the resulting change was minimal. Therefore, CaMKII-dependent enhancement of $I_{to}$ recovery from inactivation and $I_{to,slow}$ amplitude appear to be the main determinant of AP duration changes in the rabbit myocytes.

**Discussion**

The present study shows that CaMKII$\delta_C$ expression in ventricular myocytes induces alterations in both functional expression levels of $I_{to,fast}$, $I_{to,slow}$, and $I_{K1}$, as well as acute alterations in the gating of $I_{to}$ and $I_{K1}$. Only the latter effects are reversed by inclusion of CaMKII inhibitors. CaMKII$\delta_C$ reduces expression of $I_{to,fast}$ (Kv4.2/4.3) and $I_{K1}$ (Kir2.1), but increases expression of $I_{to,slow}$ (Kv1.4). CaMKII$\delta_C$ activity accelerates the recovery of $I_{to,fast}$ and $I_{to,slow}$ from inactivation and activates $I_{K1}$. There are quantitative differences in these effects in the TG mouse model vs. the rabbit overexpression model and these changes can have complex effects on the AP (prolonging the mouse APD, shortening the rabbit APD). Since CaMKII expression levels are enhanced in HF, these effects may predispose to ventricular arrhythmias.

**Separation of $I_{to}$ components and acute vs. long-term CaMKII effects**

To separate $I_{to,fast}$ and $I_{to,slow}$ components of $I_{to}$ we used the differences in time constants of recovery from inactivation (~10-30-fold) and time constants of inactivation (5-10-fold). While Kv1.4 and Kv4.2/4.3 might inactivate and recover in a multi-exponential fashion, both methods gave very similar results and the pedestal component was unchanged. This gives us confidence in the component assignments. This is further supported by the Kv1.4 and Kv4.2/4.3 expression data, which largely parallel the CaMKII$\delta_C$-induced changes in $I_{to,slow}$ and $I_{to,fast}$ amplitudes, respectively.

To distinguish between acute dynamic CaMKII-dependent regulation of $I_{to}$ and longer term effects due to altered protein expression, we used the selective CaMKII inhibitor AIP. Effects that were reversed by AIP were deemed acute (potentially mediated by CaMKII-dependent phosphorylation of channel subunits), whereas CaMKII effects that were not prevented by AIP were assumed to be longer term alterations (e.g. in protein expression).
These are functional distinctions, and complementary work is needed to define which specific CaMKII target sites are responsible for acute gating changes and longer term effects.

**CaMKII regulates $I_{to}$ functional expression**

The CaMKII$\delta_C$-induced changes in amplitudes of $I_{to,fast}$ and $I_{to,slow}$ were insensitive to AIP. This was true for mouse and rabbit myocytes and for both methods of separating $I_{to}$ components, and also for the slowing of $I_{to,slow}$ inactivation in the mouse. CaMKII$\delta_C$ overexpression enhanced $I_{to,slow}$ amplitude and Kv1.4 expression in the TG CaMKII$\delta_C$ mouse and after CaMKII$\delta_C$ overexpression in rabbit myocytes (Table 2). This suggests that the up-regulation of Kv1.4 and $I_{to,slow}$ is a relatively rapid consequence of CaMKII$\delta_C$ overexpression. While Kv1.4 protein expression was significantly enhanced, Kv1.4 mRNA changes were less significant. This raises the possibility that CaMKII might enhance trafficking and/or stability of Kv1.4 in the sarcolemma.

$I_{to,fast}$ density and Kv4.2 mRNA and protein were reduced in CaMKII$\delta_C$ TG mice, but not during short-term CaMKII$\delta_C$ overexpression in rabbit. There are several possible reasons: 1) the CaMKII$\delta_C$-dependent downregulation of $I_{to,fast}$ takes longer than 24 h to develop, 2) $I_{to,fast}$ functional downregulation depends on hypertrophy and/or HF-dependent pathways caused by CaMKII$\delta_C$ overexpression, 3) rabbit and mouse regulate functional $I_{to,fast}$ differentially. $I_{to}$ and Kv4.2/4.3 downregulation are typical in many models of hypertrophy or HF, and the fetal Kv1.4 subunit increases during hypertrophy and in HF, where CaMKII activity is increased. A remaining question is how CaMKII mediates the altered expression of channel proteins responsible for $I_{to}$.

One potential pathway by which CaMKII may regulate transcription of transient outward K-channels involves phosphorylation of type II histone deacetylases. Indeed, this system is more activated in rabbit and human HF where CaMKII is upregulated. The extent of Kv4.2 downregulation in CaMKII$\delta_C$ mice (~20%) was less than the reduction of $I_{to,fast}$ (~50%). KChIP2 is expressed in heart, and when it is co-expressed with Kv4.2 (but not with Kv1.4), it increases $I_{to}$ amplitude, slows inactivation and enhances recovery from inactivation. Here KChIP2 gene expression was reduced (by ~50%) in CaMKII$\delta_C$-TG mice. Thus, the $I_{to,fast}$ density reduction may be due to both decreases in Kv4.2 and KChIP2. Notably, chronic CaMKII inhibition in mouse was reported to downregulate KChIP2, implying that
CaMKII increases KChIP2 expression, which we do see in the rabbit experiments (Suppl Fig 1B). It is possible that the KChIP2 downregulation in TG CaMKIIδC-overexpression is secondary to the development of hypertrophic/HF signaling. This may also help explain why acute CaMKIIδC overexpression in rabbit failed to reduce Ito,fast.

**CaMKII acutely alters Ito gating**

The lack of AIP effects on Ito amplitude indicates that CaMKII does not exert acute regulation on Ito amplitude or Em-dependence of activation. However, we found that CaMKII prominently accelerates Ito,fast and Ito,slow recovery from inactivation. This suggests that CaMKII acts directly on Ito.

In HEK293 cells CaMKII-dependent phosphorylation of Ser123 of Kv1.4 channels modulates the inactivation of Ito,slow leading to an accelerated recovery from inactivation. Our study suggests that CaMKII enhances Ito,slow recovery also in rabbit and mouse myocytes.

Initial evidence for a CaMKII-dependent regulation of Ito,fast came from human atrial myocytes showing that CaMKII inhibition accelerated Ito,fast inactivation. Similar results were obtained from rat ventricular myocytes and in heterologous expression of Kv4.2/Kv4.3. It was suggested that CaMKII acts on Kv4.3 by a direct effect at Ser550, thereby prolonging open-state inactivation and accelerating the rate of recovery from inactivation. At least in vitro, CaMKII phosphorylation sites at Ser438 and Ser459 of Kv4.2 have been identified. Therefore, the CaMKII-dependent effect on Ito,fast recovery observed here may be mediated via phosphorylation of one or more of those sites.

**CaMKII regulates functional expression and regulation of IK1**

We show that the AIP-insensitive IK1 and Kir2.1 expression, were significantly downregulated in CaMKIIδC-TG mice and rabbit myocyte upon short-term CaMKIIδC overexpression (by 30-40%). So, CaMKIIδC reduces functional expression of IK1. This may partly explain the downregulation of IK1 in HF, where IK1 is down-regulated ~40-50%, and may be a consequence of chronically enhanced CaMKII activity in HF.

Acute modulation of IK1 by CaMKII seems more complex. In all cases, acute CaMKII inhibition reduces IK1 density, suggesting a constitutive CaMKII-dependent activating effect on IK1 amplitude. The overall effect of CaMKIIδC overexpression was to increase IK1 in rabbits, but to...
decrease $I_{K_1}$ in the TG mouse. We infer that in the rabbit the acute activating effect of CaMKII on $I_{K_1}$ exceeds the moderate decrease in expression induced by CaMKII$\delta_C$ overnight. In the TG mice, the reduction in $I_{K_1}$ expression may predominate over the acute activating effect of CaMKII, explaining the overall reduced $I_{K_1}$.

Heterologous Kir2.X channels are regulated by PKA, PKC and tyrosine kinases and some phosphorylation sites in the C-terminal of Kir2.1 and Kir2.3 have been confirmed biochemically. Therefore, it is possible that CaMKII may alter $I_{K_1}$ through direct channel phosphorylation.

**Cell hypertrophy**

Myocyte hypertrophy seen in CaMKII$\delta_C$-TG mice\textsuperscript{13} could dilute K-channel current density even if no changes in channel transcription per cell occurred. There was no change in rabbit ventricular myocyte membrane capacitance during the 24 h of exposure to adenovirus (Suppl. Fig. 2), ruling out this complication. For the mouse, the cellular hypertrophy (Suppl. Fig 2) would modestly decrease (<25%) K-current density (if expression/cell is unaltered). Since $I_{to,fast}$ density was reduced by ~50% and $I_{to,slow}$ density was increased in the CaMKII$\delta_C$ TG mice, this does not influence our conclusions.

**Influence of altered K-currents on APD**

CaMKII$\delta_C$ overexpression prolonged APD in mouse, but shortened APD in rabbit. This disparity may depend on differences in CaMKII-induced changes and species differences in the ionic currents. The mouse APD is short and $I_{to}$ critically determines APD.\textsuperscript{5, 41} CaMKII$\delta_C$ overexpression in mouse myocytes decreased total $I_{to}$ (Fig 1A), attributed to a large decrease of $I_{to,fast}$. At physiological heart rates in mouse $I_{to,slow}$ will have less impact than implied by Fig 1. The net reduction in $I_{to}$ may explain the prolonged APD in TG mice, although reduced $I_{K_1}$ (Fig 6A) could also contribute. CaMKII overexpression increases $I_{Na}$ and $I_{Ca}$ in mice\textsuperscript{18} which would also prolong APD, so distinguishing the relative contributions may require computer modeling.

$I_{to}$ can influence APD in the rabbit myocyte, but its influence on APD is weaker than for mouse. In rabbit myocytes overall $I_{to}$ was slightly (Fig 2A), due to an acute up-regulation of $I_{to,slow}$ (Fig 2C). This would shorten APD, and for the rabbit $I_{to,slow}$ is more likely to be relevant. In addition, the accelerated recovery from inactivation would enhance $I_{to,slow}$ availability. This would
shorten APD, as observed. The increased $I_{K1}$ could also contribute to APD shortening, while the increases in $I_{Na}$ and $I_{Ca}$ would prolong APD.

**Computational modeling**

To delineate the relative contributions of $I_{to}$ and $I_{K1}$ to the APD we used computational modeling. We incorporated the measured CaMKII-dependent alterations on $I_{Ca}$, $I_{Na}$, $I_{to}$ and $I_{K1}$ in a mouse myocyte model (see supplemental data) similar to our previous study. Equations for the fast and slow component of $I_{to}$ and a Markovian formulation of $I_{Na}$ were introduced. In TG CaMKII$\delta_C$ mouse myocytes, the reduced $I_{to,fast}$ is the dominant cause of the observed AP prolongation. While the greater $I_{Ca}$ and $I_{Na}$ did not significantly alter APD, the decreased $I_{K1}$ slightly slows the terminal repolarization making a slight contribution to the APD increase.

For rabbit, we used our previously published model incorporating CaMKII-dependent alterations on $I_{Ca}$, $I_{Na}$ and $I_{to}$. The increased rates of $I_{to}$ recovery and $I_{to,slow}$ amplitude are essential for APD shortening, whereas the changes in $I_{Na}$ and $I_{Ca}$ gating alone tend to prolong the AP duration. The addition of the increased $I_{K1}$ density caused only a few milliseconds further reduction in APD. The fact that AIP reversed the effects of CaMKII$\delta_C$ on APD in rabbit myocytes indicates that the accelerated $I_{to}$ recovery is the main determinant of the APD changes. The effect of CaMKII could potentially serve as a Ca-dependent physiologic feedback mechanism. That is, increased [Ca]$_i$ (via CaMKII) could enhance $I_{to}$ recovery, resulting in APD shortening and reduced Ca influx and [Ca]$_i$.

**Acknowledgments**

We thank Dr. Joan Heller Brown, UCSD for kindly providing the TG mice.

**Funding Sources**

Dr. Wagner was funded by the Faculty of Medicine, Georg-August-University Göttingen. Dr. Bers is supported by NIH grants (HL80101,HL30077). Dr. Maier is funded by the Deutsche Forschungsgemeinschaft (MA1982/2-1,MA1982/4-1), Deutsche Gesellschaft für Kardiologie, and Drs. Bers and Maier by the Fondation Leducq.
Conflict of Interest Disclosures

None.

References


13. Maier LS, Zhang T, Chen L, DeSantiago J, Brown JH, Bers DM. Transgenic CaMKII\deltaC overexpression uniquely alters cardiac myocyte Ca\textsuperscript{2+} handling: reduced SR Ca\textsuperscript{2+} load and activated SR Ca\textsuperscript{2+} release. *Circ Res.* 2003; 92:904-911.


Wagner et al., *CIRCULATION*HA/2008/785972

Table 1. Fitting parameters for I\textsubscript{o} recovery from inactivation

<table>
<thead>
<tr>
<th></th>
<th>Mouse [n]</th>
<th>Rabbit [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG CaMKII\delta\textsubscript{C} [6]</td>
<td>TG CaMKII\delta\textsubscript{C}+AIP [8]</td>
</tr>
<tr>
<td></td>
<td>(WT) [7]</td>
<td>(Ad-\beta\text{Gal}) [14]</td>
</tr>
<tr>
<td>(A_{\text{fast}}) (A/F)</td>
<td>1.92±0.38* (4.95±1.12)</td>
<td>2.10±0.65</td>
</tr>
<tr>
<td>(A_{\text{fast}}/y_0)</td>
<td>0.71±0.07* (0.89±0.04)</td>
<td>0.59±0.09</td>
</tr>
<tr>
<td>(k_{\text{fast}}) (s\textsuperscript{-1})</td>
<td>42.8±5.2* (12.3±2.1)</td>
<td>26.9±1.6†</td>
</tr>
<tr>
<td>(A_{\text{slow}}) (A/F)</td>
<td>1.41±0.28* (0.24±0.12)</td>
<td>1.52±0.38</td>
</tr>
<tr>
<td>(A_{\text{slow}}/y_0)</td>
<td>0.29±0.07* (0.11±0.05)</td>
<td>0.41±0.09</td>
</tr>
<tr>
<td>(k_{\text{slow}}) (s\textsuperscript{-1})</td>
<td>7.9±1.5* (1.9±0.8)</td>
<td>1.8±0.9†</td>
</tr>
<tr>
<td>(y_0) (A/F)</td>
<td>3.75±0.48 (4.90±1.29)</td>
<td>4.14±0.87</td>
</tr>
</tbody>
</table>

Fit parameters measured in mouse (average maximum \(I_{\text{o}}\) 1.04±0.13 nA, 4.16±0.57 pA/pF, \(C_m\) 260±17.5 pF, \(R_S\) 8.6±0.6 M\text{\(\Omega\)}) and rabbit (average maximum \(I_{\text{o}}\) 0.21±0.02 nA, 1.3±0.1 pA/pF, \(C_m\) 160.4±5.3 pF, \(R_S\) 8.7±0.5 M\text{\(\Omega\)}) myocytes. \(C_m=\text{membrane capacitance, } R_S=\text{series resistance.} *P<0.05 \text{ vs. } \beta\text{Gal and WT.} †P<0.05 \text{ vs. corresponding vehicle.}
Table 2. Effects of CaMKIIδC overexpression on K-currents

<table>
<thead>
<tr>
<th></th>
<th>$I_{to,fast}$</th>
<th>$I_{to,slow}$</th>
<th>$I_{K1}$ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amplitude (from recov.)</td>
<td>Ø</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>amplitude (from inact.)</td>
<td>Ø</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>amplitude (peak)</td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Protein</td>
<td>=Kv4.3</td>
<td>↑Kv1.4</td>
<td></td>
</tr>
<tr>
<td>mRNA</td>
<td>=Kv4.2/3</td>
<td>=Kv1.4</td>
<td>↓Kir2.1</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amplitude (from recov.)</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>amplitude (from inact.)</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>amplitude (peak)</td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Protein</td>
<td>↓Kv4.2/3</td>
<td>↑Kv1.4</td>
<td></td>
</tr>
<tr>
<td>mRNA</td>
<td>↓Kv4.2/3</td>
<td>↑Kv1.4</td>
<td>↓Kir2.1</td>
</tr>
<tr>
<td>Both</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{to}$ amplitudes unaltered by AIP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{to}$ inactivation kinetics unaltered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{to,fast}$ and $I_{to,slow}$ recover faster from inactivation (acutely AIP-sensitive)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: Maximal currents, protein data, and mRNA levels were derived from I-V-curves, Western blots, and RT-PCR results, respectively. ↑=upregulation; ↓=downregulation; Ø=unaltered. *with AIP, which reduces $I_{K1}$ in all cases.
Figure Legends

Figure 1
I_{to} current-voltage relation in mouse myocytes. (A) Mean data of total I_{to} which was significantly reduced in TG. (B) Original records. (C) I_{to,slow} amplitude was enhanced in TG but not reversed with AIP. Time constant of I_{to,slow} inactivation was slowed (right), but not reversed by AIP. (D) The reduction of total I_{to} was based on a smaller I_{to,fast} in TG and not influenced by AIP. The time constant of I_{to,fast} inactivation was not altered (right). (E) Steady-state currents were not affected. Average maximum I_{to}: 1.85±0.15 nA, 7.82±0.77 pA/pF, C_{m} 257±18.6 pF, R_{S} 8.5±0.6 MΩ. *P<0.05 vs. WT (ANOVA).

Figure 2
I_{to} current-voltage relation in rabbit myocytes. (A) Mean data of total I_{to}. (B) Original records. (C) The increase of total I_{to} appears to be due to an increase of I_{to,slow} amplitude and not reversible by AIP, (D) while I_{to,fast} was not altered. Unchanged time constants of I_{to,slow} and I_{to,fast} inactivation (right panels). (E) Steady-state currents were not affected. Average maximum I_{to}: 0.32±0.03 nA, 2.12±0.16 pA/pF, C_{m} 151±4.8 pF, R_{S} 8.6±0.4 MΩ. *P<0.05 vs. βGal (ANOVA).

Figure 3
I_{to} recovery from inactivation in mouse myocytes. (A) Protocol. (B) Mean data for total I_{to}. Inset shows I_{to} recovery within the first 2 s. Data was fit to a double exponential function (Table 1). (C) Original records. I_{to} recovery from inactivation was enhanced in TG vs. WT (*P<0.05, F-test) which was reversible with AIP (†P<0.05, F-test).

Figure 4
(A) I_{to} recovery from inactivation in rabbit myocytes. Mean data for total I_{to}. *P<0.05 vs. βGal; †P<0.05 vs. CaMKIIδC (F-test) (B+C) Recovery rate constants and amplitudes of I_{to,fast} and I_{to,slow}. (B) I_{to,fast} and I_{to,slow} where significantly enhanced after CaMKII overexpression in rabbit and mouse myocytes and could be slowed using AIP. (C) After adenoviral CaMKIIδC overexpression, the amplitude of I_{to,slow} was significantly increased. I_{to,fast} was unchanged. In TG mice, I_{to,slow} amplitude was increased, but I_{to,fast} was significantly reduced. All effects were unaffected upon
CaMKII inhibition. *P<0.05 vs. WT or βGal; †P<0.05 vs. corresponding vehicle (one-way ANOVA).

**Figure 5**

K-channel expression. (A) Western blots of Kv1.4 and Kv4.2 from mouse heart. (B) Western blots of Kv1.4 and Kv4.3 in rabbit myocytes. (C+D) Confocal microscopy using immunocytological stainings of CaMKII, Kv1.4 (C), Kv4.3 (D). Merge indicates the close relationship of CaMKII with Kv1.4 and Kv4.3. *P<0.05 (t-test)

**Figure 6**

I_{k1} current-voltage relation. (A) Mean data for peak I_{k1} in myocytes from TG mice (vs. WT) and rabbit CaMKIIδC myocytes (vs. βGal). *P<0.05 vs. WT and βGal; †P<0.05 vs. TG CaMKIIδC and CaMKIIδC; #P<0.05 vs. WT+AIP and βGal+AIP (ANOVA). (B) Original traces. Each upper trace is the raw current, each mid trace shows the remaining current after BaCl₂, and each lower trace depicts the BaCl₂-sensitive current used for analysis.

**Figure 7**

AP measurements. (A) Original traces at 0.25 Hz in mouse and rabbit myocytes. Insets show simulated traces at 0.25 Hz. (B+C) Mean data of APD at 60% (APD60) and 80% (APD 80) for 0.25-2 Hz. (B) Compared to WT, APDs were prolonged in TG; AIP did not influence APDs. (C) APDs were shorter in CaMKIIδC-overexpressing rabbit myocytes vs. βGal; AIP re-lengthened APDs.
Figure 2

A

Total $I_{to}$ (A/F)

$-$60  $-$40  $-$20   0   20   40   60

Voltage (mV)

$\beta$Gal, N=21  CaMKII$_{C}$, N=22
$\beta$Gal+AIP, N=12  CaMKII$_{C}$+AIP, N=11

B

WT

200 pA

100 ms

TG CaMKII$_{C}$

200 pA

100 ms

C

$I_{to,slow}$ (A/F)

$-$60  $-$40  $-$20   0   20   40   60

Voltage (mV)

D

$I_{to,fast}$ (A/F)

$-$60  $-$40  $-$20   0   20   40   60

Voltage (mV)

E

$I_{ss}$ (A/F)

$-$60  $-$40  $-$20   0   20   40   60

Voltage (mV)
Figure 4

A

Total $I_{to} (P_2/P_1)$

\[ \text{Time (s)} \]

Rabbit

- $\beta$Gal, $N=14$
- CaMKII\(\delta\)c, $N=13$
- CaMKII\(\delta\)c+AIP, $N=8$

B

Fast Recovery

- $k_{fast}$ for recovery (s\(^{-1}\))
- Rabbit: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP, WT, CaMKII\(\delta\)c+AIP
- Mouse: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP

C

Fast Amplitude

- $I_{to,fast}$ (A/F)
- Rabbit: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP, WT, CaMKII\(\delta\)c+AIP
- Mouse: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP

Slow Recovery

- $k_{slow}$ for recovery (s\(^{-1}\))
- Rabbit: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP, WT, CaMKI\(\delta\)c+AIP
- Mouse: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP

Slow Amplitude

- $I_{to,slow}$ (A/F)
- Rabbit: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP, WT, CaMKI\(\delta\)c+AIP
- Mouse: $\beta$Gal, CaMKII\(\delta\)c, CaMKI\(\delta\)c+AIP

Downloaded from http://circp.ahajournals.org/ by guest on April 29, 2016
Figure 5

A

Mouse

Kv1.4/GAPDH (normalized)

WT N=8

TG CaMKIIδ, N=8

100 kD

75 kD

37 kD

Kv1.4

GAPDH

B

Rabbit

Kv1.4/GAPDH

βGal, N=15

CaMKIIδ, N=16

100 kD

75 kD

37 kD

Kv1.4

GAPDH

C

D

CaMKIIδ

Kv 1.4

merge

1

2

3

10 μm

CaMKIIδ

Kv 4.3

merge

1

2

3

10 μm
Figure 6

A  
Mouse

![Graph showing voltage (mV) vs. I_K1 (A/F) for different conditions in mouse.](http://circep.ahajournals.org/)

- WT, N=3
- WT+AIP, N=3
- TG CaMKIIδC, N=5
- TG CaMKIIδC+AIP, N=6

Rabbit

![Graph showing voltage (mV) vs. I_K1 (A/F) for different conditions in rabbit.](http://circep.ahajournals.org/)

- βGal, N=10
- βGal+AIP, N=5
- CaMKIIδC, N=9
- CaMKIIδC+AIP, N=6

B

![Current traces for different conditions in mouse and rabbit.](http://circep.ahajournals.org/)

- WT
- TG
- TG CaMKIIδC
- CaMKIIδC+AIP
- βGal
- CaMKIIδC
- CaMKIIδC+AIP

Downloaded from http://circep.ahajournals.org/ by guest on April 29, 2016
Ca/Calmodulin Kinase II Differentially Modulates Potassium Currents
Stefan Wagner, Elena Hacker, Eleonora Grandi, Sarah L. Weber, Nataliya Dybkova, Samuel Sossalla, Thomas Sowa, Larissa Fabritz, Paulus Kirchhof, Donald M. Bers and Lars S. Maier

Circ Arrhythm Electrophysiol. published online April 17, 2009;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/early/2009/04/17/CIRCEP.108.842799

Data Supplement (unedited) at:
http://circep.ahajournals.org/content/suppl/2009/07/09/CIRCEP.108.842799.DC1.html

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

METHODS

Patch-clamp experiments

Ruptured-patch whole cell voltage clamp was used to measure \( I_{lo} \), \( I_{k1} \), and membrane potential \( (E_m) \), respectively. For \( I_{lo} \) and \( I_{k1} \) measurements, microelectrodes (2-3 M\( \Omega \)) were filled with (mmol/l) 110 K-aspartate, 20 KCl, 1 MgCl\(_2\), 5 Mg-ATP, 0.3 Li-GTP, 10 HEPES, 5 EGTA, 1.8 CaCl\(_2\) (free [Ca] =100 nmol/l), pH 7.2 (KOH). In order to block Ca-activated chloride currents, 0.01 mmol/l niflumic acid was added to the pipette solution. The bath solution contained (in mmol/l) 135 tetramethylammonium chloride as a substitute for Na, 4 KCl, 1 MgCl\(_2\), 10 glucose, 2 CaCl\(_2\) (1 for mouse), 10 HEPES, (pH 7.4, KOH). Ca channels were blocked with 0.3 mmol/l CdCl\(_2\), 0.01 mmol/l atropine was used to prevent muscarinic receptor activation, and 0.1 \( \mu \)mol/l thapsigargin was added to the external solution to block Ca uptake into the sarcoplasmic reticulum (SR). Fast capacitance which is generated largely by the pipette itself was compensated in cell-attached configuration. Liquid junction potentials (usually 3-6 mV) were corrected. Membrane capacitance \( (C_m) \) and series resistance \( (R_s) \) were compensated after patch rupture; access resistance was typically <10 M\( \Omega \). After each recording of \( I_{k1} \), cells were superfused with bath solution containing 500 mmol/l BaCl\(_2\) to block \( I_{k1} \), the resulting trace was then subtracted from the original trace to determine the Ba-sensitive current.

For \( E_m \) measurements, glass microelectrodes of >10 M\( \Omega \) resistance when filled with pipette solution filled with (mmol/l) 120 K-aspartate, 8 KCl, 7 NaCl, 1 MgCl\(_2\), 10 HEPES, 5 Mg-ATP, 0.3 Li-GTP (pH 7.2, KOH) were used. The bath solution contained (mmol/l) 140 NaCl, 4 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\) (1 for mouse), 10 glucose, 5 HEPES (pH 7.4, NaOH). Access resistance was typically ~20 M\( \Omega \) after patch rupture. Liquid junction potentials (~4-7 mV) were corrected. APs were elicited by square current pulses of 1-2 nA amplitude and 1-5 ms duration at basic cycle lengths of 0.5, 1, 2, 4 s. A steady-state AP was considered the last of a train of 20 at the same stimulation rate. All recordings were done 5 min after rupture. Signals were filtered with 2.9 and 10 kHz Bessel filters and recorded with an EPC10 amplifier (HEKA Elektronik). All experiments were conducted at room temperature.

To inhibit CaMKII autocamtide 2-related inhibitory peptide (AIP, Sigma-Aldrich) was added to the pipette solution (0.1 mmol/l).

Data analysis and statistics

All data are expressed as mean±S.E.M. To analyse \( I_{lo,fast} \) and \( I_{lo,slow} \), we made use of their difference in the kinetic of recovery and inactivation. The recovery from inactivation was fit to a double exponential:

\[
y(t) = A_{fast} \left(1 - \exp\left(-k_{fast}t\right)\right) + A_{slow} \left(1 - \exp\left(-k_{slow}t\right)\right) + y_0,
\]

where \( y(t) \) is the current at time \( t \), \( A_{fast} \) and \( A_{slow} \) are the current amplitudes, \( k_{fast} \) and \( k_{slow} \) are the rate constants of recovery, and \( y_0 \) is the plateau current. \( I_{lo} \) decay as a measure of inactivation was fit to a double exponential:

\[
y(t) = A_{fast} \exp\left(-k_{fast}t\right) + A_{slow} \exp\left(-k_{slow}t\right) + y_{ss},
\]

where \( y(t) \) is the current at time \( t \), \( A_{fast} \) and \( A_{slow} \) are the current amplitudes, \( k_{fast} \) and \( k_{slow} \) are the reciprocals of the inactivation time constants \( \tau_{fast} \) and \( \tau_{slow} \), and \( y_{ss} \) is the steady-state current \( I_{ss} \). When applicable, curve fits were tested for significant difference using the F-test. For longitudinal data, two-way repeated measures ANOVA was run; else Student’s unpaired t test or one-way ANOVA with Dunnetts multiple comparison was used. For all other multiple comparisons, the significance level was adjusted according to the Bonferroni method. P-values of P<0.05 were considered significant.

Western blot analysis

Transfected ventricular rabbit myocytes were harvested and lysed in Tris buffer (mmol/l) 20 Tris-HCl, 200 NaCl, 20 NaF, 1 Na\(_3\)VO\(_4\), 1% Triton X-100, 1 DTT (pH 7.4) and complete protease inhibitor cocktail (Roche Diagnostics) by trituration. Whole mouse hearts were homogenized in...
Tris buffer. Protein concentration was determined by BCA assay (Pierce Biotechnology). Denatured cell lysates and tissue homogenates (30 min, 37°C in 2% β-mercaptoethanol) were subjected to Western blotting (10% SDS-polyacrylamide gels) using a rabbit polyclonal Anti-KV 1.4, anti-KV 4.3 anti-KV 4.2 (1:500, Alomone Labs) as primary and a horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG (1:10000, Amersham Biosciences) as secondary antibody.

**Immunocytochemical staining**

Myocytes were washed (PBS), fixed (100% methanol, 15 min, -20°C), and blocked with 1% BSA (overnight, 4°C). Cells were incubated with primary antibodies (monoclonal mouse anti-HA, Roche Diagnostics, 1:100, rabbit polyclonal Anti-KV 1.4, 1:150, anti-KV 4.3 1:75 Chemicon, anti-KV 4.2 1:60, Alomone Labs in 0.5% BSA and 0.5% Triton X-100, overnight, 4°C). After washing (PBS), incubation with secondary antibodies (Alexa Fluor 555 goat anti-mouse IgG, Alexa Fluor 488 goat anti-rabbit IgG, 1:200, Molecular Probes) was done in 0.5% BSA (2h, room temperature). Cells were covered with Vectashield Hard Set mounting medium (Vector Laboratories) and viewed using a confocal microscope. For control, no primary antibodies were used.

**RT-PCR**

Fluorescence-based kinetic real-time PCR was performed using a Bio-Rad iQ5 Real-Time PCR Detection System. Total RNA was isolated from isolated mouse and rabbit ventricular myocytes using the Qiagen RNeasy kit with on-column DNase digestion. First-strand cDNA synthesis was done using the Quanta qScript cDNA SuperMix kit and realtime PCR amplification was performed using the Quanta PerfeCTa SYBR Green SuperMix for iQ. The primers (Suppl. Table 1) were obtained from Eurofins MWG. Triple assays were used. For every amplification of the DNA template of interest (TOI), a corresponding amplification of GAPDH was used as internal control. The cycle threshold (CT) was determined after background fluorescence was substrated and fluorescence intensity was converted to the log scale. The threshold was set as the minimal cycle value separating the amplified template signal from noise. Relative expression values were calculated as $2^{-(CT \text{ TOI} - CT \text{ GAPDH})}$. These values were then normalized for mean control (WT or βGal) to be 1. Two-sided t-tests were only applied to the raw $2^{-ΔCT}$ values. P<0.05 was considered significant.

**Mouse action potential simulation**

We have included the previously published data on CaMKII-dependent alterations on $I_{\text{Ca}}$ and $I_{\text{Na}}$ and the novel data on $I_{\text{lo}}$ and $I_{\text{K1}}$ reported here in the mouse ventricular AP model developed by Demir. The formulation is based on the rat right ventricular cell model previously published by the same group on the template of their rat left ventricular myocyte model. The description of intracellular Ca dynamics includes a restricted subspace located between the sarcoplasmic reticulum and T tubules into which Ca enters though L-type and is release by RyR channels. With respect to the original formulation, we incorporated the equations describing the fast and slow components of $I_{lo}$ and a markovian formulation of $I_{\text{Na}}$ as in the Bondarenko model of the mouse ventricular AP. Simulations and parameter identification were performed using Simulink (The MathWorks. Inc- Natick, Mass; USA). Pacing was simulated by a current pulse train (pulses of 5 ms in duration) of 0.6 pA in amplitude with different frequencies (0.25, 0.5, 1 and 2 Hz). A variable order solver based on the numerical differentiation formulas was used to numerically solve the model equations (ode15s). The stimulation was maintained until a steady AP was reached (typically ~50 s).

$I_{\text{Na}}$ was simulated with a Markov model, which structure (Suppl. Fig. 3) is based on that proposed by Clancy and Rudy and implemented in Grandi et al to account for acute CaMKII overexpression effects. Suppl. Table 3 shows the expressions of the transitions rates between the Markov model states. The corresponding parameters were identified by a fitting procedure to
simulate the electrophysiological characterization of cardiac $I_{Na}$ in WT and in TG (CaMKIIδC overexpressing) mouse myocytes reported by Wagner et al.\textsuperscript{4} as described in Grandi et al.\textsuperscript{12} A full list of the parameters is in Suppl. Table 4.

The L-type Ca current formulation of Pandit et al.\textsuperscript{7} was modified to reproduce the effects of CaMKII\textsuperscript{2}, by increasing the channel maximal conductance by 10% and by mimicking Ca-dependent facilitation with an additional term that makes the Ca-dependent inactivation less complete. To account for CaMKII overexpression, the Ca dependent inactivation variable becomes:

$$Ca_{\text{inact}} + Ca_{\text{inact,CaMKII}}$$

where

$$Ca_{\text{inact,CaMKII}} = \frac{1}{1 + (0.01/[Ca]_{ss})}$$

and [Ca]\textsubscript{ss} is the calcium concentration (in mM) in the restricted cleft subspace.

The formulations of the slow and fast component of $I_{to}$ were adapted from Bondarenko et al.\textsuperscript{10} to account for the increased expression levels of K\textsubscript{v}1.4 and reduced expression levels of K\textsubscript{v}4.2/4.3 channel proteins and for the enhanced recovery from inactivation in myocytes from CaMKII-transgenic mice with respect to WT. To this purpose, the maximal conductance of the slow component $G_{to,slow}$ was increased by 50% and $G_{to,fast}$ was reduced to one third in the CaMKII model. The time constant of $I_{to,fast}$ and $I_{to,slow}$ inactivation were modified to fit the recovery from inactivation data as in Suppl. Table 5.

Equations describing $I_{K1}$ were implemented as in the original formulation, and $I_{K1}$ conductance was reduced by 50% to reproduce CaMKII overexpression data.

**RESULTS**

The adequacy of the Na\textsuperscript{+} channel Markovian model is confirmed by the comparison between experimental and simulated voltage-clamp data (Suppl. Fig. 4). As in the experiments, chronic CaMKIIδC overexpression shifts inactivation to more negative potentials with respect to WT (Suppl. Fig. 4A), without affecting $I_{Na}$ activation (Suppl. Fig. 4B, WT and TG-CaMKIIδc traces are superimposed). In the computer simulations, as in the experimental recordings, intermediate inactivation is enhanced by CaMKIIδC (Suppl. Fig. 4C) and recovery from inactivation is slower (Suppl. Fig. 4D). Along with these loss-of-function effects, upon prolonged depolarization simulated CaMKIIδC overexpression resulted in a larger late $I_{Na}$ (Suppl. Fig. 4E) as experimentally observed. Transgenic CaMKIIδc overexpression in mouse ventricular myocytes decreases total $I_{to}$ amplitude and speeds recovery from inactivation, and the effects are well reproduced in silico (Suppl. Fig. 5).

**REFERENCES**

### SUPPLEMENTAL TABLES

**Supplement Table 1. RT-PCR Primers**

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Species</th>
<th>Forward 5'</th>
<th>Reverse 5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.4</td>
<td>Mouse</td>
<td>CAT TTG GTT TCC CAA TGG TC 3'</td>
<td>GTG GTG CAT TCC TTG TTC CT 3'</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>Mouse</td>
<td>GCC GCA GCA CCT AGT CGT T 3'</td>
<td>CAC CAC GTC GAT GAT ACT CAT GA 3'</td>
</tr>
<tr>
<td>KChIP2</td>
<td>Mouse</td>
<td>GGC TGT ATC ACG AAG GAG GAA 3'</td>
<td>CCG TCC TTG TTT CTG TCC ATC 3'</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>Mouse</td>
<td>AAG AGC CAC CTT GTG GAA GCT 3'</td>
<td>CCT TCT GAA GTG ATC GTA GTT TTG AGA 3'</td>
</tr>
<tr>
<td>Kv1.4</td>
<td>Rabbit</td>
<td>CAC CGA CAG AGC GGC TTT 3'</td>
<td>CTC CCT TAG GAT CTC CTC CTC AGA 3'</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>Rabbit</td>
<td>TCG GAG CTG GGC TTC TTG 3'</td>
<td>CGT AGA ACA TCA CTG TAG CAA AGA TG 3'</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>Rabbit</td>
<td>AGG AGT ACA AGG ACC GCA AGA G 3'</td>
<td>GGT TGT TCT CCG AGT CGT TGT C 3'</td>
</tr>
<tr>
<td>KChIP2</td>
<td>Rabbit</td>
<td>TTG TCG GTG ATT CTT CGG GGA A 3'</td>
<td>CTA GAT GAC ATT GTC AAA GAG C 3'</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>Rabbit</td>
<td>AAT GCA GAC TTT GAA ATT GTT 3'</td>
<td>CTC TGG CAC TAC ACA GGG 3'</td>
</tr>
</tbody>
</table>
### Supplement Table 2. Measured and simulated APD<sub>60</sub> and APD<sub>80</sub> in mouse and rabbit ventricular myocytes at varying pacing frequency

<table>
<thead>
<tr>
<th>(Hz)</th>
<th>Mouse [n]</th>
<th>Rabbit [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Simulated</td>
</tr>
<tr>
<td>APD 60 (ms)</td>
<td>APD 80 (ms)</td>
<td>APD 60 (ms)</td>
</tr>
<tr>
<td>0.25</td>
<td>18.0±2.6* (9.7±1.0)</td>
<td>33.7±3.8* (18.1±2.5)</td>
</tr>
<tr>
<td>0.5</td>
<td>23.4±3.0* (14.6±2.7)</td>
<td>58±20.6* (31.1±5.4)</td>
</tr>
<tr>
<td>1</td>
<td>28.3±7.3* (15.7±2.7)</td>
<td>69±26.5* (30.6±8.6)</td>
</tr>
<tr>
<td>2</td>
<td>26.2±4.1* (14.4±2.4)</td>
<td>54±10.3* (31.8±8.5)</td>
</tr>
</tbody>
</table>

Legend: *P<0.05 vs. WT or βGal, respectively, †P<0.05 vs. corresponding vehicle
### Supplement Table 3. Transition rate expressions (ms⁻¹)

<table>
<thead>
<tr>
<th>Transition rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_1 = \frac{P_{1a1}}{P_{2a1} \exp\left(-\frac{V_m + 2.5}{17}\right) + 0.20 \exp\left(-\frac{V_m + 2.5}{150}\right)} )</td>
</tr>
<tr>
<td>( a_2 = \frac{P_{1a1}}{P_{2a1} \exp\left(-\frac{V_m + 2.5}{15}\right) + 0.23 \exp\left(-\frac{V_m + 2.5}{150}\right)} )</td>
</tr>
<tr>
<td>( a_3 = \frac{P_{1a1}}{P_{2a1} \exp\left(-\frac{V_m + 2.5}{12}\right) + 0.25 \exp\left(-\frac{V_m + 2.5}{150}\right)} )</td>
</tr>
<tr>
<td>( b_1 = P_{1b1} \exp\left(-\frac{V_m}{P_{2b1}}\right) )</td>
</tr>
<tr>
<td>( b_2 = P_{1b2} \exp\left(-\frac{V_m - P_{2b2}}{P_{2b1}}\right) )</td>
</tr>
<tr>
<td>( b_3 = \frac{P_{1b3} \exp\left(-\frac{V_m - P_{2b3}}{P_{2b1}}\right)}{P_{2b1}} )</td>
</tr>
<tr>
<td>( a_5 = P_{1a5} \exp\left(-\frac{V_m + 7}{P_{2a5}}\right) )</td>
</tr>
<tr>
<td>( b_5 = \frac{P_{1b5} + P_{2b5} (V_m + 7)}{P_{2b5}} )</td>
</tr>
<tr>
<td>( a_4 = 1 \left(\frac{P_{1a4} \exp\left(-\frac{V_m + 7}{P_{2a4}}\right)}{P_{2a4}} + P_{3a4}\right) )</td>
</tr>
<tr>
<td>( b_4 = \frac{a_3 \ a_4 \ a_5}{b_3 \ b_5} )</td>
</tr>
<tr>
<td>( a_6 = a_4 / P_{1a6} )</td>
</tr>
<tr>
<td>( b_6 = P_{1b6} \exp\left(-\frac{V_m}{P_{2b6}}\right) )</td>
</tr>
<tr>
<td>( a_7 = (P_{1a7} \exp(V_m/P_{2a7})) )</td>
</tr>
<tr>
<td>( b_7 = P_{1b7} \exp(-V_m/P_{2b7}) )</td>
</tr>
<tr>
<td>( a_8 = P_{1a8} )</td>
</tr>
<tr>
<td>( b_8 = P_{1b8} )</td>
</tr>
</tbody>
</table>
Supplement Table 4. Na\(^+\) channel model parameters for WT and TG-CaMKII\(\delta_c\) channels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WT</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{1a1}</td>
<td>3.802</td>
<td></td>
</tr>
<tr>
<td>P_{2a1}</td>
<td>0.1027</td>
<td></td>
</tr>
<tr>
<td>P_{1a4}</td>
<td>0.188495</td>
<td></td>
</tr>
<tr>
<td>P_{2a4}</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>P_{3a4}</td>
<td>0.393956</td>
<td></td>
</tr>
<tr>
<td>P_{1a5}</td>
<td>7e^{-7}</td>
<td></td>
</tr>
<tr>
<td>P_{2a5}</td>
<td>7.2</td>
<td>7.8</td>
</tr>
<tr>
<td>P_{1b1}</td>
<td>0.1917</td>
<td></td>
</tr>
<tr>
<td>P_{2b1}</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>P_{1b2}</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>P_{2b2}</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>P_{1b3}</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>P_{2b3}</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>P_{1b5}</td>
<td>0.0044</td>
<td>0.00467</td>
</tr>
<tr>
<td>P_{2b5}</td>
<td>2e^{-5}</td>
<td></td>
</tr>
<tr>
<td>P_{1a6}</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>P_{1b6}</td>
<td>8.9554e^{-7}</td>
<td>6.5449e^{-7}</td>
</tr>
<tr>
<td>P_{2b6}</td>
<td>11.3944</td>
<td></td>
</tr>
<tr>
<td>P_{1a7}</td>
<td>0.487e^{-4}</td>
<td>0.3377e^{-3}</td>
</tr>
<tr>
<td>P_{2a7}</td>
<td>23.2696</td>
<td></td>
</tr>
<tr>
<td>P_{1b7}</td>
<td>0.2868e^{-3}</td>
<td>1.868e^{-4}</td>
</tr>
<tr>
<td>P_{2b7}</td>
<td>35.9898</td>
<td></td>
</tr>
<tr>
<td>P_{1a8}</td>
<td>0.1e^{-7}</td>
<td>6.5e^{-6}</td>
</tr>
<tr>
<td>P_{1b8}</td>
<td>9.8e^{-3}</td>
<td>3.8e^{-3}</td>
</tr>
</tbody>
</table>
Supplement Table 5. $I_{to,fast}$ and $I_{to,slow}$ inactivation time constants (ms) for WT and TG-CaMKIIδC channels

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{to,fast}$</td>
<td>$\tau_{ii,f} = 2e \left( \frac{V_m + 45}{2} \right) + 0.03$</td>
<td>$\tau_{ii,f} = 1.7e \left( \frac{V_m + 55}{26} \right)^2 + 0.03$</td>
</tr>
<tr>
<td>$I_{to,slow}$</td>
<td>$\tau_{ii,s} = 200 + \frac{2000}{1 + e^{\frac{V_m + 45}{10.7}}}$</td>
<td>$\tau_{ii,s} = 1005e \left( \frac{V_m + 45}{240} \right)^2 + \frac{500}{1 + e^{-V_m - 45}}$</td>
</tr>
</tbody>
</table>
Supplement Figure 1

A Mouse

Supplement Figure 1

A Mouse

B Rabbit

Relative mRNA expression patterns measured via RT-PCR are shown. A) Relative expression values for Kv1.4, Kv4.2, KChIP2 and Kir2.1 in isolated ventricular myocytes obtained from CaMKIIδC-transgenic mice or WT littermates are shown. Although not statistically significant, there was a strong trend towards an increased m-RNA expression of Kv1.4 in TG vs. WT (P=0.1). B) Relative expression values for Kv1.4, Kv4.2, Kv4.3, KChIP2 and Kir2.1 in isolated rabbit ventricular myocytes acutely overexpressing CaMKIIδC vs. βGal are depicted. * - P<0.05 (t-test)
Membrane capacitance as a measure of cellular hypertrophy for isolated ventricular myocytes obtained from CaMKIIδC-transgenic mice (vs. WT, left panel), and isolated rabbit ventricular myocytes transfected with adenovirus encoding for CaMKIIδC (vs. β-Gal, right panel). * - P<0.05 (t-test)

Markov model of the cardiac Na⁺ channel. The channel model contains background (upper nine states) and burst (lower four states) gating modes. The burst mode reflects a population of channels that transiently fail to inactivate and accounts for a sustained inward current (or late current).
Supplement Figure 4

(A) Steady-state inactivation. CaMKIIδC overexpression shifts the availability curve towards negative potentials. (B) Activation. Na⁺ channel activation is not affected by CaMKIIδC overexpression (traces are superimposed). CaMKIIδC enhances the intermediate inactivation (C) and slows the recovery from inactivation (D). (E) Late I_{Na} is enhanced by CaMKIIδC overexpression. Left, simulated traces. Right, bar graphs of simulated and experimental normalized current integrals. Experiments are from Wagner et al (2006). * P<0.05
Supplement Figure 5

CaMKII effects on $I_{to}$. (A) Representative current traces under voltage clamp activation protocol for simulated CaMKII mediated $I_{to}$. (B) Simulated I-V relations for total $I_{to}$. CaMKII\(\delta_C\) (red) mediated decrease of the total current (vs. WT, black) is mainly due to CaMKII\(\delta_C\) effects on the fast component. (C) Recovery from inactivation was investigated using a 500 ms depolarization pulse (from -80 mV holding potential to +50 mV) followed by recovery intervals of increasing durations and a subsequent test pulse. Recovery from inactivation is significantly increased by CaMKII\(\delta_C\). Symbols represent experimental data, lines simulation results for WT (black) and TG-CaMKII\(\delta_C\) (red).