Phosphatidylinositol 4,5-bisphosphate (PIP2) is necessary for the function of various ion channels. The potassium channel, \( I_{Ks} \), is important for cardiac repolarization and requires PIP2 to activate. Here we show that the auxiliary subunit of \( I_{Ks} \), KCNE1, increases PIP2 sensitivity 100-fold over channels formed by the pore-forming KCNQ1 subunits alone, which effectively amplifies current because native PIP2 levels in the membrane are insufficient to activate all KCNQ1 channels. A juxtamembranous site in the KCNE1 C terminus is a key structural determinant of PIP2 sensitivity. Long QT syndrome associated mutations of this site lower PIP2 affinity, resulting in reduced current. Application of exogenous PIP2 to these mutants restores wild-type channel activity. These results reveal a vital role of PIP2 for KCNE1 modulation of \( I_{Ks} \) channels that may represent a common mechanism of auxiliary subunit modulation of many ion channels.

**Results**

**KCNE1 Slows the PIP2-Dependent Channel Rundown.** To investigate the regulation of \( I_{Ks} \) by PIP2, we expressed KCNQ1 with and without KCNE1 in oocytes from *Xenopus laevis* and recorded currents using patch-clamp and two-electrode voltage-clamp techniques. Currents recorded from inside-out membrane patches expressing KCNQ1 immediately and rapidly decayed following patch excision such that channel activity was completely lost by PIP2 coexpression with KCNE1. This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1100872108/-/DCSupplemental.
lost within 5 min (Fig. 1A). This rundown of current was fit well with a single exponential (Fig. 1B). However, after excising inside-out membrane patches expressing KCNQ1 + KCNE1, currents remained stable over 3 min prior to rundown, which was not observed with KCNQ1 alone (Fig. 1B). The subsequent rundown of KCNQ1 + KCNE1 current was slower than that observed with KCNQ1 alone (Fig. 1 B and C). This rundown of I_{Ks} activity is attributed to the loss of PIP2 from the excised membrane patch (6). The PIP2 dependence of current rundown after patch excision was supported by the following experiments. The voltage sensitive phosphatase from *Ciona intestinalis* (GIVSP) that dephosphorylates PIP2 upon membrane depolarization (10) was coexpressed with KCNQ1 + KCNE1. GIVSP hastens PIP2 loss from the membrane patch, resulting in a faster rate of current rundown. Conversely, directly applying 10 μM of exogenous PIP2 to the intracellular face of membrane patches expressing KCNQ1 + KCNE1 prevented current rundown for longer than 10 min (Fig. 1 B and C). These results show that PIP2 loss after patch excision leads to loss of channel activity.

KCNQ1 activity is lost more quickly after patch excision than KCNQ1 + KCNE1, indicating that KCNE1 slows PIP2-dependent rundown. To further demonstrate this effect of KCNE1, we injected oocytes with the same amount of KCNQ1 mRNA and various amounts of KCNE1 mRNAs in molar ratios of 1:0, 1:0:01, 1:0:05, and 1:1. The activation kinetics and the steady-state voltage dependence of activation of the expressed channels depend on the KCNQ1:KCNE1 ratio, which suggested that pore-forming KCNQ1 subunits are associated with varying numbers of KCNE1 subunits (11–13) (Fig. S1). The time course of current rundown became progressively longer with higher concentrations of KCNE1 due to a longer delay and a larger time constant of rundown (Fig. 1D). A lower molar ratio of KCNQ1:KCNE1 mRNA results in a larger portion of the expressed channel population containing a higher stoichiometry of KCNE1:KCNQ1, and these results show that channels with more KCNE1 subunits have slower PIP2-dependent rundown (Fig. 1 D and E).

Why does the association of KCNE1 affect the time course of PIP2-dependent rundown? To answer this question, we applied PIP2 to the intracellular face of membrane patches after complete current rundown, which restored channel activity in a dose-dependent manner (Fig. 2A). The effective PIP2 concentrations of half maximal activation (EC50) for KCNQ1 and KCNQ1 + KCNE1 are >600 μM and 4.6 μM, respectively (Fig. 2 B and C). These results show that KCNE1 greatly enhances the PIP2 sensitivity of the channel resulting in a longer time course of rundown as the channels can still function at lower PIP2 levels. To further confirm the correlation between time course of PIP2-dependent rundown and PIP2 sensitivity, we studied two LQT mutations of KCNQ1, R539W, and R555C, coexpressed with KCNE1, both of which decrease PIP2 sensitivity (Fig. 2 B and C) (5). Consistently, these mutants shortened the time course of rundown by eliminating the delay and decreasing the time constant of rundown relative to WT KCNQ1 + KCNE1 (Figs. 1C and 2B).

It is important to note that application of high levels of PIP2 could increase the current of KCNQ1 beyond the level measured immediately following patch excision (Fig. 2A and B). This result suggests that the native PIP2 level in the patch membrane is not sufficient to saturate the PIP2 binding and activation of all KCNQ1 channels; therefore, supernormal levels of exogenous PIP2 can activate channels that are PIP2 unbound in the native membrane patch. In contrast, high doses of PIP2 could not increase the current of KCNQ1 + KCNE1 beyond the amplitude immediately following patch excision (Fig. 2 B and D), indicating that KCNE1 association enhances PIP2 affinity, such that channel activation by PIP2 is saturated immediately following patch excision. This conclusion is supported by the delay before PIP2-dependent current rundown that is present only when KCNE1 is coexpressed with KCNQ1 (Fig. 1 B–E). This delay likely represents a period when the PIP2 level in the patch membrane is supersaturating for activating KCNQ1 + KCNE1. Rundown occurs when the PIP2 level falls below a threshold of saturation and channels become nonfunctional due to unbinding of PIP2. These results suggest that KCNE1 coexpression increases the current amplitude by recruiting KCNQ1 channels that would be nonfunctional due to a lack of PIP2 binding.

**Key Structural Determinants of PIP2 Sensitivity in KCN1 Proteins**

*Proteins associate with PIP2 through electrostatic interactions between basic residues and the negatively charged head group of PIP2 (4, 14). Application of diC4-PIP2 and diC8-PIP2, which have different fatty acid tail groups as the PIP2 purified from cell membrane (see Materials and Methods) has the same effect on KCNQ1 + KCNE1 currents (Fig. 2B), supporting that the PIP2 head group is important in interactions with the channel protein. In order to identify the key structural determinants in KCN1 contributing to enhanced PIP2 sensitivity, we made mutations that individually neutralized each of the 11 basic residues located in the cytosolic C terminus of KCNE1 and measured the time course of rundown after patch excision for each mutant coexpressed with KCNQ1. Of the 11 neutralizing mutations, 4 (R67Q, K69C, K70C, and H73N) abolished the delay and significantly reduced the time constant of rundown, indicating that neutralization of these basic residues leads to a decreased PIP2 sensitivity (Fig. 3 A and B). In the KCN1 solution NMR structure in micelles (15), these residues are positioned on a helical stretch at the bottom of the transmembrane domain (Fig. 3C). The α-helicity of the juxtamembranous portion of the KCNE1 C terminus is also supported by mutagenic perturbation analysis (16). The basic residues at positions 67, 69, 70, and 73 lie on one face of the α-helix, which provide a dense cluster of positive charges that would be attractive for the negatively charged head group of PIP2.

Our mutational study cannot definitively identify these residues as a PIP2 binding site because the mutations could affect PIP2 binding through an allosteric mechanism. However, the clustering of these basic residues juxtaposed with the inner leaflet of the plasma membrane suggests that they could directly interact with PIP2. Consistent with this idea, a triple charge-reversal mu-
tation, R67E/K69E/K70E, renders the time course of rundown ever faster than that of KCNQ1 alone (Fig. 3 A and B), as if PIP2 were repulsed by the negative charges.

**PIP2 Restores the Loss of Function Due to LQT Mutations in KCNE1**

Mutations of the putative PIP2 interaction site in KCNE1 (R67C, R67H, K70M, and K70N) have been previously identified in LQT patients as disease-associated mutations (7–9). We find that coexpressing the KCNE1 mutation with KCNQ1 decreases current amplitude and shifts the voltage dependence of activation toward more depolarized potentials compared to WT KCNQ1 + KCNE1 channels (Fig. 4 A–C). Each of these changes in channel properties would decrease the contribution of IKS to termination of cardiac action potentials, resulting in prolongation of action potential duration and creating a substrate for potentially fatal arhythmias. The PIP2–dose-response curves of KCNQ1 + R67C and KCNQ1 + K70N (Fig. 4 D) are significantly right-shifted in comparison to that of WT KCNQ1 + KCNE1, indicating that these LQT-associated mutations decrease PIP2 sensitivity of the channel complex. Furthermore, the current amplitude measured by applying saturating levels of PIP2 exceeded the current amplitude immediately following patch excision, suggesting that a reduction of PIP2 affinity decreases the number of PIP2-bound channels in the native membrane resulting in less current. Remarkably, application of a saturating concentration of PIP2 (300 μM) to the intracellular face of membrane patches expressing KCNQ1 + mutant KCNE1 restored the wild-type channel current characteristics. Specifically, the current amplitude increased 2- to 5-fold (Fig. 4 E), accounting for all of the reduction in the whole cell current of the mutant channels (Fig. 4 A and C). Additionally, the voltage dependence of channel activation shifted back toward less depolarized voltages to nearly superimpose with that of the WT KCNQ1 + KCNE1 (Fig. 4 F). These results show that a decrease in PIP2 sensitivity of the Ikβ channel due to these KCNE1 mutations can lead to LQT syndrome.

**Discussion**

Ion channel β-subunits modulate pore-forming α-subunits, increasing the diversity of ion channel properties that can be generated from a limited repertoire of genes encoding α- and β-subunits. To understand the function of ion channels in vivo requires structure-function knowledge of not only the pore-forming subunits, but also their heteromeric complexes with β-subunits. The interactions between KCNQ1 and KCNE1 have been studied intensely for many years as this pair of α- and β-subunits assembles in the cardiac myocyte and generates the Ikβ current that is important in the termination of cardiac action potentials. However, despite these efforts, the molecular mechanisms by which KCNE1 changes the KCNQ1 channel to generate the Ikβ current remain elusive. In this work we show that KCNQ1 increases the PIP2 sensitivity of Ikβ by several orders of magnitude (Fig. 2). Because of the low PIP2 sensitivity of KCNQ1, the PIP2 level in biological membrane is too low to saturate binding of KCNQ1, leaving a fraction of the channel population nonfunctional in the absence of KCNE1. This unique mechanism of current amplification is independent of KCNE1 effects on single channel con-
ductance for which previous reports have provided conflicting results (17–19).

In this study we identify four residues (R67, K69, K70, and H73) in proximal C terminus of KCNE1 as key determinants of IP2 sensitivity (Fig. 3). Previous studies have highlighted the KCNE1 C terminus as critical for the modulation of IKS current characteristics. A C-terminal deleted KCNE1 mutant assembled with KCNQ1, but did not modify current properties (20). Functional scan of KCNE1 point mutations revealed that the juxtamembranous C-terminal region of KCNE1 is especially intolerant to mutation, indicating that this region likely plays an important role in controlling channel activity (21). Docking of the NMR structure of KCNE1 to a KCNQ1 homology model indicates that the proximal C terminus of KCNE1 may participate in intimate interaction with the S4–S5 linker suggesting that KCNE1 could directly influence the channel gating machinery (15). The colocalization of the KCNE1 proximal C terminus and the gating machinery of KCNQ1 have also been shown experimentally by the formation of disulfide bonds between pairs of cysteine mutations engineered in the KCNE1 C terminus and the S4–S5 linker or the bottom of S6 (22). Interestingly, basic residues in the bottom of S6 and in the S4–S5 linker have been proposed to interact with IP2 (5). Purified fragments of the KCNQ1 proximal C terminus demonstrated broad binding of anionic lipids in biochemical assays that depended on several juxtamembranous basic residues in KCNQ1 (23). These results indicate that the key determinants of IP2 sensitivity in KCNE1 (residues R67, K69, K70, and H73) reside in the same region that is critical for KCNE1 modulation of IKS and interaction with the gating machinery. Thus, IP2 may be an integral component in the same channel complex, and the interactions among IP2, KCNE1, and KCNQ1 may hold the key to resolve the long-standing absence of a mechanistic understanding of how KCNE1 shapes the IKS channel function.

Mutations of the key residues in KCNE1 that are determinants of IP2 sensitivity, R67C, R67H, K70M, and K70N, are associated with long QT syndrome (7). These mutations reduce IKS current and IP2 sensitivity (Fig. 4). Remarkably, we are able to rescue wild-type channel characteristics by applying supernormal levels of exogenous IP2, suggesting that the disease pathogenesis of these mutations can be explained by a decrease in IP2 sensitivity of the channel complex. IP2 levels can be changed in the cardiac myocyte by activation of phospholipase C (PLC) via G-protein coupled receptors, such as α1-adrenergic receptors and M1-muscarinic receptors, that are coupled to Gq/PLC hydrolyzes IP2 to generate diacylglycerol and inositol 1,4,5-trisphosphate, which lead to protein kinase C (PKC) activation and increased intracellular calcium, respectively (4). The generation of second messengers in addition to the effective decrease of IP2 levels makes IKS modulation by PLC activation complex as IP2 is known to be sensitive to PKC phosphorylation (24), intracellular calcium (25), and IP2 (6). This complex regulation in response to activation of Gqα may be reflected in the biphasic response of heterologously expressed IKS channels (26) and the conflicting reports of effects on endogenous IKS in myocytes (27–29). Our current results show that the IP2 sensitivity of the IKS channel is dependent on the KCNQ1 expression level relative to that of KCNQ1 (Fig. 1), suggesting a possibility that KCNE1 may modulate the response to PLC activation. Further studies with the consideration of this possibility may provide previously undescribed insights on the effects of the Gqα signaling pathway on IKS channels.

IKs channels are also modulated by β-adrenergic receptor stimulation due to the phosphorylation of residues S27 and S92 in KCNQ1 by protein kinase A (PKA) (30, 31), which increases IKS currents and shortens ventricular action potentials. Importantly, this phosphorylation of KCNQ1 alters channel function only with KCNE1 coexpression (32), indicating that, similar to IP2 modulation, the KCNE1 interaction with KCNQ1 is critical for the PKA-dependent modulation. A previous study suggested a cross-talk between PKA and IP2 modulations of the IKS channel (31). To examine whether phosphorylation of IKS channel affects IP2-dependent modulation, we studied the properties of the mutation S27D/S92D in KCNQ1 coexpressed with KCNE1, which has been shown to mimic the effects of PKA phosphorylation of IKS channels (31, 32). Consistent with previous results, we found that S27D/S92D affects IKS function, including a shift of voltage-dependent activation to more negative voltages and slowing of deactivation kinetics (Fig. 5A–C). However, the phosphomimetic mutation S27D/S92D does not alter the time course of IP2-dependent rundown of the IKS current (Fig. 5D), suggesting that PKA phosphorylation does not alter IP2 modulation. Likewise, the slowing of deactivation caused by the phosphomimetic mutation is not dependent on the time after patch excision during which IP2 levels decrease (Fig. 5E), suggesting that IP2 does not affect the functional changes caused by PKA phosphorylation. These results suggest that, if a cross-talk between PKA and IP2 modulations of the IKS channel exists, it does not happen in the channel protein.

The KCNE family of ion channel β-subunits contains five family members that have been reported to modulate the activity of a variety of channel α-subunits in ion channel complexes. Many of these channel α-subunits or channel complexes are also modulated by IP2 (Table 1). Sequence alignment shows that the basic residues that are essential for KCNE1 modulation of IKS, IP2 sensitivity are highly conserved across all members of the KCNE family of peptides (Fig. 6), suggesting that modulation of IP2 sensitivity may be a common mechanism of current modulation by the KCNE β-subunits. Interestingly, some members of the KCNE family may modulate more than one channel α-subunit. Likewise, multiple KCNE family members may modulate the same channel α-subunit (Table 1). Because of structural differ-
Table 1. • channel α-subunits that interact with KCNE families and show PIP₂ sensitivity

<table>
<thead>
<tr>
<th>KCNE</th>
<th>α-subunit*</th>
<th>PIP₂ sensitivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNE1</td>
<td>KCNQ1 (1, 2)</td>
<td>α + β (6)</td>
</tr>
<tr>
<td></td>
<td>HERG (33)</td>
<td>α (34)</td>
</tr>
<tr>
<td>Kv 4.3 (35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNE2</td>
<td>KCNQ1 (36)</td>
<td>α + β (37)</td>
</tr>
<tr>
<td></td>
<td>KCNQ2 and 2/3 (38)</td>
<td>α (39)</td>
</tr>
<tr>
<td></td>
<td>HERG (40)</td>
<td>α (34)</td>
</tr>
<tr>
<td></td>
<td>HCN1&amp;2 (41)</td>
<td>α (42)</td>
</tr>
<tr>
<td>Kv4.2 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kv4.3 (35)</td>
<td></td>
</tr>
<tr>
<td>KCNE3</td>
<td>KCNQ1 (44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kv3.1 and Kv3.1 (45)</td>
<td>α (46)</td>
</tr>
<tr>
<td></td>
<td>Kv3.4 (47)</td>
<td>α (48)</td>
</tr>
<tr>
<td></td>
<td>HERG (44)</td>
<td>α (34)</td>
</tr>
<tr>
<td></td>
<td>KCNQ4 (44)</td>
<td>α (49)</td>
</tr>
<tr>
<td>KCNE4</td>
<td>KCNQ1 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KV1.1 and Kv1.3 (51)</td>
<td>α (48)</td>
</tr>
<tr>
<td>KCNE5</td>
<td>KCNQ1 (52)</td>
<td></td>
</tr>
</tbody>
</table>

*Coexpression of a KCNE with the α-subunit changes the properties of the channel.
†PIP₂ sensitivity of the channel either with the α-subunits alone (α) or with the α-subunits and a KCNE coexpression (α + β) has been reported.

ences in these α- and β-subunits, the putative interactions among KCNE β-subunits, channel α-subunits and PIP₂ may vary within different ion channel complexes, resulting in diverse effects of KCNE peptides.

Materials and Methods

Mutagenesis and Oocyte Preparation. KCNQ1 and KCNE1 were subcloned into pcDNA3.1(+)(Invitrogen). All mutations were generated by using overlap extension PCR and verified by sequencing. mRNA was transcribed in vitro by using the mMessage mMachine T7 polymerase kit (Applied Biosystems). The full-length cells were removed by using type 1A collagenase (Sigma-Aldrich).

Stage IV-V Xenopus oocytes were selected and injected with 4.6 ng mRNA per oocyte. Injected oocytes were incubated in ND96 solution (in mM: 96 NaCl, 2 KC1, 1.8 CaCl₂, 1 MgCl₂, and 5 Hepes, pH 7.60) at 18 °C for 3–5 d before recording.

Electrophysiology

Macroscopic currents were recorded from inside-out patches formed with patch pipettes of 0.5–1.0 MΩ resistance. The data were acquired using an Axopatch 200-B patch-clamp amplifier (Axon Instruments) and pulse acquisition software (HEKA). Experiments were conducted at 22 °C. The pipette solution contained (in mM) 140 KMeSO₄, 20 Hepes, 2 KC1, and 2 MgCl₂, pH 7.2. The internal solution contained (in mM) 140 KMeSO₄, 20 Hepes, 2 KC1, 5 EGTA, 1.5 MgATP, and PIP₂ as a


Fig. S1. Channel properties with different ratios of KCNQ1 to KCNE1. (A) Superimposed current traces recorded from 4 oocytes with KCNQ1: KCNE1 mRNA ratios of 1:1 (open circle); 1:0.05 (open triangle); 1:0.01 (open square); 1:0 (filled circle). The currents were elicited by a depolarizing voltage pulse to 40 mV from a holding potential of −80 mV. (B) Normalized G-V curves for different ratios of KCNQ1: KCNE1, 1:1 (open circles), \( V_{1/2} = 21.2 \pm 2.2 \text{ mV} \); 1:0.05 (open triangles), \( V_{1/2} = 8.8 \pm 2.2 \text{ mV} \); 1:0.01 (open squares), \( V_{1/2} = -9.3 \pm 1.5 \text{ mV} \); 1:0 (filled circles), \( V_{1/2} = -15.2 \pm 2.9 \text{ mV} \). \( n = 6 \) for all experiments.