Abstract—Nearly a hundred different KCNQ1 mutations have been reported as leading to the cardiac long QT syndrome, characterized by prolonged QT interval, syncopes, and sudden death. We have previously shown that phosphatidylinositol-4,5-bisphosphate (PIP2) regulates the KCNQ1–KCNE1 complex. In the present study, we show that PIP2 affinity is reduced in three KCNQ1 mutant channels (R243H, R539W, and R555C) associated with the long QT syndrome. In giant excised patches, direct application of PIP2 on the cytoplasmic face of the three mutant channels counterbalances the loss of function. Reintroduction of a positive charge by application of methanethiosulfonate ethylammonium on the cytoplasmic face of R555C mutant channels also restores channel activity. The channel affinity for a soluble analog of PIP2 is decreased in the three mutant channels. By using a model that describes the KCNQ1–KCNE1 channel behavior and by fitting the relationship between the kinetics of deactivation and the current amplitude obtained in whole-cell experiments, we estimated the PIP2 binding and dissociation rates on wild-type and mutant channels. The dissociation rate of the three mutants was higher than for the wild-type channel, suggesting a decreased affinity for PIP2. PIP2 binding was magnesium-dependent, and the PIP2-dependent equilibrium constant in the absence of magnesium was higher with the wild-type than with the mutant channels. Altogether, our data suggest that a reduced PIP2 affinity of KCNQ1 mutants can lead to the long QT syndrome.

Key Words: KCNQ1 • KCNE1 • phosphatidylinositol-4,5-bisphosphate • long QT syndrome

KCNQ1 is the pore-forming subunit of the channel complex (composed of KCNQ1 and its regulator KCNE1) underlying the delayed rectifier potassium current $I_{Ks}$, a key modulator of the action potential duration in the human heart.1,2 Nearly 100 KCNQ1 mutations have been reported to cause the long QT syndrome, a genetic disease characterized by prolonged cardiac repolarization, cardiac arrhythmias, and a high risk of sudden death.3,4

Phosphatidylinositol-4,5-bisphosphate (PIP2) is an important intracellular regulator of many ion channels and transporters.5–8 We showed recently that intracellular PIP2 regulates KCNQ1–KCNE1 channel activity in such a way that PIP2 stabilizes the open state of the channels, leading to an increased current amplitude, slowed deactivation kinetics, and a shift in the activation curve toward negative potentials.9 In general, it is known that positively charged amino acids are implicated in channel–PIP2 interactions.10–13 Because various KCNQ1 mutations producing neutralization of a residue in an intracellular segment of the channel protein lead to reduced current amplitude, accelerated deactivation, and shift in the activation curve toward positive potentials,14,15 we hypothesized that mutations neutralizing a positive charge (eg, R243H, R539W, and R555C) may act through an impaired channel–PIP2 interaction. We thus compared the effects of PIP2 on wild-type and mutant KCNQ1 channel behavior using the patch-clamp technique. We observed that intracellular PIP2 rescued the mutated channel abnormal activity. Moreover, application to the R555C mutant of methanethiosulfonate ethylammonium (MTSEA), which mimics a positive charge by its association with cysteine 555, reverses the loss of function phenotype, suggesting that a positive charge at this position is critical for normal KCNQ1 activity. This is consistent with the electrostatic nature of the channel–PIP2 interactions.6,12,16 We also show that the channel affinity for a soluble analog of PIP2 is decreased in the three mutant channels. Finally, we used our previously developed computer model of KCNQ1–KCNE1 channel activity9 to further characterize the mutant channel behavior. We found that an alteration in PIP2 binding in mutants R539W and R555C fully explains the channel dysfunction. For mutant R243H, the data suggested an effect of the mutation not only on PIP2 binding but also on voltage sensing.
DMEM (GIBCO). Cells were transfected with the plasmids complexed with Fugene-6 (Roche Molecular Biochemical) according to the standard protocol recommended by the manufacturer. The R243H, R539W, and R555C mutations were subcloned from pCI KCNQ1 plasmids (a kind gift of Dr Jacques Barhanin, Institut de Pharmacologie Moleculaire et Cellulaire, Valbonne, France) into the human pCDNA3.1 KCNQ1–KCNE1 concatemer17 (human KCNE1 linked to the N terminus of the human KCNQ1; a kind gift of Dr Robert S. Kass, Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, NY). The plasmid coding for the green fluorescent protein (pEGFP) that we used to identify transfected cells was purchased from Clontech. For giant patch experiments, relative DNA composition was 80% pCDNA3.1 KCNQ1–KCNE1 and 20% pEGFP (of a total of 2 μg of DNA). For whole-cell experiments, relative DNA composition was 40% pCDNA3.1 KCNQ1–KCNE1 and 60% pEGFP (of a total of 2 μg of DNA).

**Electrophysiology**

Twenty-four to 72 hours after transfection, COS-7 cells were mounted on the stage of an inverted microscope and constantly perfused at a rate of 2 mL/min. The bath temperature was maintained at 22.0 ± 1.0°C. Stimulation, data recording, and analysis were performed by Acquis1, a software made by Gérard Sadoc (distributed by Bio-logic Science Instruments) through an analog-to-digital converter (Tecmar TM100 Labmaster; Scientific Solution). Electrodes were connected to a patch-clamp amplifier (RK-300; Bio-logic Science Instruments). For giant patch experiments, the procedure described by Couey et al18 was adapted to realize giant patches from COS-7 cells as follows. An “excision” pipette, filled with the standard solution, was connected to a 20-mL syringe to apply suction for excision. Pipettes were pulled from borosilicate glass capillaries (glass type 8250; Garner Glass) on a vertical puller (P30; Sutter Instruments) and fire-polished using a microforge (MF-83; Narishige) to reach 9- to 15-μm tip diameters for patch pipettes and 20 μm

![Figure 1. KCNQ1–KCNE1 concatemer channels behave like independent subunits. A, Representative whole-cell recordings of wild-type (WT) or mutant KCNQ1–KCNE1 concatemers. A step protocol of four depolarizations was applied every 14 s. D1 to D4 are as indicated above each current trace and were adapted to the voltage sensitivity of the channel. B, Activation curves of wild-type and mutant KCNQ1–KCNE1 concatemers calculated from the tail current measured after each depolarization on recordings shown in A. Data are fitted by a Boltzmann equation. C, Left, White bars are wild-type or mutant channel $V_{0.5}$ obtained from the coexpression of independent subunits.14,15 Black bars are $V_{0.5}$ for wild-type or mutant KCNQ1–KCNE1 concatemers (n = 7 to 18). Middle, White bars are deactivation time constant (taudeact) of wild-type or mutant current obtained from the expression of independent subunits.14,15 Black bars are wild-type or mutant KCNQ1–KCNE1 concatemer current taudeact measured after the last depolarization step to D4 (n = 7 to 20). Right, White bars are k of wild-type or mutant activation curve obtained from the expression of independent subunits.14,15 Black bars are k of wild-type or mutant KCNQ1–KCNE1 concatemer activation curve (n = 6 to 18).
for excision pipettes. Cells were continuously perfused with the standard solution. A microperfusion system allowed local application and rapid change of the different experimental solutions. KCNQ1–KCNE1 currents were investigated with a protocol consisting of depolarized voltage steps of 1 s from a holding potential of −80 mV to +40 mV and then to −40 mV for 500 ms, every 5 s. Because it was difficult to maintain isolated patch at potential more positive than 80 mV, step protocols were performed in the whole-cell configuration. Channel deactivation kinetics were obtained by using a monoexponential fit.

For whole-cell experiments, micropipettes (tip resistance 2 to 3 MOhm) were pulled from thin-walled glass (Kimble). For the half-maximal activation potential (V_{0.5}), currents, and deactivation measurements, the membrane potential was stepped, from a holding potential (−80 mV) to four voltage steps adapted to V_{0.5} of the wild-type and mutant channels (Figure 1) and then stepped back to −40 mV, where tail currents are visible. The activation curve was fitted to a Boltzmann distribution. KCNQ1–KCNE1 deactivation kinetics were obtained by a monoexponential fit.

Patch-clamp data are presented as mean±SEM. Statistical significance of the observed effects was assessed by Student’s t tests. Off-line analysis was performed using Acquis1 and Microsoft Excel programs. Microsoft Solver was used to fit data by a least-square algorithm.

Kinetic Model of PIP_{2} Regulation of KCNQ1–KCNE1

Fits of τ_{deact} versus maximum open probability (P_{o}; or the maximal tail current amplitude in Figure 6A) were generated from the model presented in Figure 6B. In the following model, P_{o max} is the maximal P_{o} for a given membrane PIP_{2} level (P_{o max} tends toward 1 when PIP_{2} increases). Only PIP2 binding rate constant (k_{PIP2}) is PIP_{2} dependent.

\[ \tau_{deact} = -2/[((k_{PIP2} + k_{s1})/4/k_{s1} - k_{s1})^{0.5} - k_{PIP2} - k_{s1}] \]

and

\[ k_{PIP2} = k_{s2} P_{o max}/(1 - P_{o max}) \]

Solutions and Drugs

For giant patch experiments, cells were perfused with a standard solution containing (in mmol/L): 140 KCl, 10 HEPES, and 1 EGTA, pH 7.3 with KOH. The following solution (in mmol/L): 145 K-gluconate, 10 HEPES, and 1 EGTA, pH 7.3 with KOH, was used to perfuse the cell during K^+ currents measurement and to fill the

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Figure 2. PIP_{2} partially corrects the loss of function related to R243H, R539W, and R555C mutations. A, Representative giant inside-out patch recordings of wild-type (WT) or mutant KCNQ1–KCNE1 currents in symmetrical potassium before (control) and 2 minutes after patch excision in the presence of 1.4 mmol/L MgATP (0.6 mmol/L free Mg^{2+}) and 5 μmol/L PIP_{2}. For comparison, the wild-type current in control condition is shown in gray. The voltage protocol as shown was applied every 5 s. B, Current amplitude of wild-type or mutant channels measured at the end of the depolarizing step (I_{40 mV}) plotted against time. Excision was conducted at time zero. Current amplitudes in control conditions (a) and 2 minutes after patch excision (b) are indicated by vertical dotted lines (WT n=19; R243H n=19; R539W n=22; R555C n=16). C, Wild-type or mutant channel current amplitude in conditions a and b from B. *P<0.05 vs wild-type.
patch pipette. For whole-cell experiments, the pipette (intracellular) solution used had the following composition (in mmol/L): 150 KCl, 10 HEPES, and 5 EGTA, pH 7.2 with KOH. The standard Tyrode perfusion solution contained (in mmol/L): 145 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, and 5 HEPES, pH 7.4 with NaOH. Free activities were calculated using a software designed by G.L. Smith (University of Glasgow, Scotland). PIP₂ (Roche Molecular Biochemical) was sonicated on ice for 30 minutes before application to inside-out patches. 1,2-dioctanoyl (diC₈)–PIP₂ was purchased from Cayman Chemicals. MTSEA was purchased from TRC. All other products were purchased from Sigma.

Results

KCNQ1–KCNE1 Concatemers Behave Like the Independent Subunits

In our previous study,⁹ we investigated the regulation of the KCNQ1–KCNE1 complex by making use of the inside-out configuration of the patch-clamp technique. Sizable currents were recorded in only 15% of the patches. To improve the success rate of inside-out recordings, we increased the size of the patches and adopted the giant patch configuration.¹⁸ Using large pipettes, we achieved gigaOhm seals in 87% of total attempts and obtained 73.5% of giant patches with sizable currents (on a total of 238 attempts). We also used a construct of hKCNQ1 fused to hKCNE1¹⁷ (KCNQ1–KCNE1) to prevent variability caused by variable KCNQ1–KCNE1 expression ratio.

We validated the use of the KCNQ1–KCNE1 concatemer by checking that the fusion did not interfere with the wild-type or mutant channel properties. Using the whole-cell configuration, we obtained values for $V_{0.5}$, slope factor (k), and deactivation kinetics for the concatemer similar to those previously obtained by Chouabe et al¹⁴,¹⁵ for the independent subunits (Figure 1).

PIP₂ Partially Corrects the Loss of Function Related to R243H, R539W, and R555C Mutations

We hypothesized that if the loss of function associated with R243H, R539W, and R555C was caused by a lower PIP₂ sensitivity, application of PIP₂ in excess to the intracellular side of the mutant channel should counterbalance the deficit in sensitivity. A total of 5 μmol/L PIP₂ is beyond physiological levels because this concentration increases the open stability of ATP-sensitive potassium channels ($K_{ATP}$) and

Figure 3. The loss of function associated with R555C is partially corrected by 1 mmol/L MTSEA. A, Representative inside-out recordings of wild-type (WT) or R555C mutant KCNQ1–KCNE1 currents before (control) and after excision and then PIP₂ and after 1 mmol/L MTSEA application. For comparison, the wild-type current trace in control condition is represented in gray; same voltage protocol as in Figure 2. B, Wild-type or mutant channel $I_{-40 mV}$ plotted against time (gray box, before; black box, during; hatched box, after 1 mmol/L MTSEA application). C, Normalized activating current amplitude and deactivation time constant (τ_{deact}) of wild-type (n=8) and R555C mutant (n=8) channels before and after MTSEA application. *P<0.05 vs PIP₂ condition values.
KCNQ1–KCNE1 channels. In giant patches, we monitored the activity of wild-type and mutant channels during application of 5 μmol/L PIP2 plus 1.4 mmol/L MgATP (MgATP was required to prevent the PIP2-independent rundown). Before excision, R243H, R539W, and R555C channels generated current amplitudes significantly smaller than wild-type channels (Figure 2). In particular, it was difficult to obtain sizable currents from R555C mutant channels. On excision in high PIP2 plus MgATP, wild-type and mutant KCNQ1–KCNE1 channel currents increased. For each construct, current amplitudes before and 2 minutes after excision statistically differed. Most importantly, PIP2 application restored the current amplitude of the mutant channels to values similar to the wild-type control (Figure 2B and 2C).

Loss of Function Associated With R555C Is Partially Corrected by 1 mmol/L MTSEA

The latter experiments cannot discriminate the effects related to charge neutralization from the effects related to other changes in the amino acid chain (length, addition of an aromatic ring). With mutation R555C, the cysteine residue can be modified by sulfhydryl reagents, some of which are positively charged. Such a reagent, MTSEA applied to Kir2.1 cysteine mutants restored their PIP2 sensitivity. In giant patches, we monitored the activity of wild-type and mutant channels during application of 5 μmol/L PIP2 plus 1.4 mmol/L MgATP (MgATP was required to prevent the PIP2-independent rundown). Before excision, R243H, R539W, and R555C channels generated current amplitudes significantly smaller than wild-type channels (Figure 2). In particular, it was difficult to obtain sizable currents from R555C mutant channels. On excision in high PIP2 plus MgATP, wild-type and mutant KCNQ1–KCNE1 channel currents increased. For each construct, current amplitudes before and 2 minutes after excision statistically differed. Most importantly, PIP2 application restored the current amplitude of the mutant channels to values similar to the wild-type control (Figure 2B and 2C).

Loss of Function Associated With R555C Is Partially Corrected by 1 mmol/L MTSEA

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Loss of Function Associated With R555C Is Partially Corrected by 1 mmol/L MTSEA

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excised patches, application of MTSEA had no effect on the wild-type KCNQ1–KCNE1 current amplitude but increased the R555C current 5.9±2.4-fold (Figure 3; n=8). This effect was irreversible, remaining after washing out MTSEA. Application of MTSEA had no significant effect on wild-type current kinetics but significantly decelerated R555C deactivation (Figure 3C). This suggests that as in Kir1.1 and Kir2.1 channels, a neutralization of an amino acid side chain in KCNQ1 channels is responsible for the decreased electrostatic interaction with PIP₂.

**R243H, R539W, and R555C Mutants Have a Lower Affinity for diC₈–PIP₂ Than Wild-Type Channels**

The weak water solubility of PIP₂ prevents the investigation of its dose-dependent effects on KCNQ1 channels and therefore characterization of the channel affinity for PIP₂. To circumvent this difficulty, previous studies used a water-soluble PIP₂ analog with short acyl chains. Using the same approach, we tested the application of 1 to 100 μmol/L of a short chain PIP₂ analog, diC₈–PIP₂ on wild-type and mutant KCNQ1–KCNE1 channels in the giant patch configuration. The PIP₂ analog was applied after the spontaneous PIP₂-dependent rundown. The EC₅₀ for diC₈–PIP₂ activation was increased in the three mutants compared with wild-type (Figure 4), further suggesting a decreased PIP₂ affinity. The Hill coefficient was 1.3 for wild-type channels and close to 1 for the three mutants, suggesting that several PIP₂-binding sites act cooperatively and that cooperativeness decreases in the three mutant channels.

**Unmasking PIP₂ Charges by Magnesium Dilution Leads to an Increased Current Amplitude and a Slowing in Deactivation**

Giant patch experiments suggested a decreased affinity for PIP₂ of the mutants. However, alternate hypotheses cannot be excluded: (1) mutations may decrease the channel activity independently of the PIP₂ regulation (ie, PIP₂ binding site is not altered but channel PIP₂ affinity is indirectly modified). Such an allosteric effect has been reported previously for regulation by ATP of K₁,A channels; (2) in the giant patch configuration, we used MgATP because the KCNQ1–

![Figure 5. Unmasking of PIP₂ charges by magnesium dilution leads to an increase in wild-type current amplitude and a slowing in deactivation. A, Top, Voltage protocol. Four depolarization steps were applied every 14 s to monitor the V₀.5. Bottom, Representative whole-cell recording of wild-type (WT) KCNQ1–KCNE1 concatemer currents 28 (a), 112 (b), and 198 s (c) after patch rupture, with a magnesium-free pipette solution. B, Normalized tail currents recorded after the last depolarization step showing the slower deactivation after patch rupture. C, Plot of the tail current at −40 mV recorded after the last depolarization step (Iₜₐₕₜₐₜ, tau deact, and V₀.5 as a function of time after patch rupture.)
KCNE1 activity required PIP2 and MgATP. It is possible that the presence of free Mg2+ in the solution may screen the negative charges of PIP2 and limit PIP2 effect on channel activity; and the current density cannot be accurately measured in giant patches because the capacitance of the patch is too small. This prevented investigation of the consequence of the mutations on the functional channel density.

We therefore used the whole-cell configuration and omitted Mg2+ in the pipette. This led to an increased availability of PIP2, because of gradual intracellular Mg2+ dilution with the pipette milieu. A representative recording of wild-type KCNQ1–KCNE1 is shown in Figure 5. A four–depolarization step protocol was used to estimate maximum activation (Pmax) as a function of time after patch rupture. Deactivation kinetics were measured during repolarization to −40 mV. As shown in Figure 5A and 5C, the maximal tail current measured during repolarization (I tail) increased after rupture of the patch (a to b). After a delay, the deactivation time constant also increased dramatically (c; from 583±74 to 1911±653 ms; n=20). This was not observed in the presence of Mg2+ in the pipette (when measured 150 s after the patch rupture, taudeact evolved from 430±46 to 381±70 ms; n=5; data not illustrated). Fitting the relationship between taudeact and maximal tail current amplitude (I tail; shown for 3 representative cells in Figure 6A) by using the equation obtained from the kinetic model presented in Figure 6B provided additional information. As shown in Figure 6A, the PIP2 dissociation rate can be calculated (kPIP2=1/taudeact at 0 current amplitude), as well as the maximum current amplitude (Imax), which corresponds to the current when available PIP2 is maximal and is proportional to the number of channels present in the membrane patch. The PIP2 binding rate can also be calculated, which varied during Mg2+ dilution: k<sub>PPP</sub>=k<sub>PPP</sub>*I<sub>control</sub>/I<sub>max</sub> (1−I<sub>control</sub>/I<sub>max</sub>). The inferred k<sub>PPP</sub> variation (Figure 6C) suggested that the observed modification of the current amplitude and deactivation were attributable to an increase in the channel–PIP2 interaction, which was reduced by intracellular magnesium before the patch rupture.

**Molecular Dissection of the Effect of R243H, R539W, and R555C on KCNQ1–KCNE1 Channel Activity**

The same experiments were repeated using mutants R243H, R539W, and R555C. A four-step protocol was applied with voltages adapted to the mutant channel V<sub>a</sub> (Figure 1, legend). To compare the different mutants, I<sub>−40 mV</sub> was normalized using its maximum value calculated as in Figure 6A (I<sub>−40 mV</sub>/I<sub>max</sub> =P<sub>a</sub> max). Plotting taudeact as a function of P<sub>a</sub> max (Figure 7A and 7B) indicated that k<sub>PPP</sub> was greater with the three mutants.

Because the maximal current density (I<sub>max</sub>) was not significantly different between wild-type and mutant channels (Figure 7D), we deduced that trafficking of mutant channel was not altered in our cells. A further analysis of the taudeact relation to P<sub>a</sub> max brought additional information on the channel characteristic. We calculated the PIP2 binding rate (k<sub>PPP</sub>) from the taudeact versus P<sub>a</sub> max relationship (Figure 7C). As with the wild-type channels, the mutant k<sub>PPP</sub> increased as a function of time after patch rupture, suggesting an unmasking of PIP2-negative charges during Mg2+ dilution. The equilibrium constant (k<sub>PPP</sub>/k<sub>PPP</sub>) in the absence of
magnesium was smaller for every mutant compared with wild-type, suggesting a net decrease in the PIP₂ affinity for R243H, R539W, and R555C (Figure 7D).

Using the model, we calculated $k'_{S4}$ for wild-type and mutant channels from the equation linking $\tau_{deact}$ to $k_{PIP2}$, $k'_{PIP2}$, and $k'_{S4}$ (see Materials and Methods). Only R234H $k'_{S4}$ differed from wild-type $k'_{S4}$ (Figure 7D). This alteration in S4 properties is consistent with the position of the mutation (next to S4) and the change in the slope factor ($k$) of the activation curve.\(^\text{14}\) The change in $k$ observed with R243H cannot be attributed to the decreased PIP₂ affinity because the R539W and R555C mutants showed a similar decrease in PIP₂ affinity as R243H (Figure 4) but no change in $k$. We conclude that the R243H mutation induces a decreased PIP₂ affinity and a modification of the movement of the S4 segments, the latter inducing a more pronounced loss of function through faster transitions of S4 from permissive to nonpermissive states.

**Discussion**

The present work shows that several long QT mutations are associated with a decreased PIP₂ sensitivity of the KCNQ1–KCNE1 channel complex. For R243H, an alteration in the voltage sensor was also detected. The R243H mutation has been identified in a proband that displayed a typical Jervell and Lange–Nielsen syndrome, a recessive syndrome that associates deafness and a long QT phenotype.\(^\text{15}\) The R539W and R555C mutations were identified in patients with a dominantly inherited classical long QT (Romano–Ward) syndrome.

We used a combination of patch-clamp experiments and modeling to dissect the effects of the three mutations on the channel activity. The $\tau_{deact}$ versus $P_o$ max plots provided estimation of three major parameters of the channel activity: the channel density represented by $I_{max}$. 

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**Figure 7.** A molecular dissection of the effect of mutations R243H, R539W, and R555C on the KCNQ1–KCNE1 channel complex activity. A, Superimposed plots of $\tau_{deact}$ vs normalized $I_{40mV}$ ($P_o$ max=1--$I_{40mV}$/Imax) (wild-type [WT] n=19; R243H n=11; R539W n=7; R555C n=7). B, Representative plots of $\tau_{deact}$ vs $P_o$ max for wild-type and mutant channels. C, Calculated $k_{PIP2}$ as a function of time after patch rupture (same cells as in B). Lines, Single exponential fit of $k_{PIP2}$. D, Bar graphs summarizing all the parameters obtained from the above experiments. $I_{max}$ (corresponding to the current when available PIP₂ is maximal) and $k_{PIP2}$ calculated as in Figure 6A. $k_{PIP2}/k_{PIP2}$ at the patch rupture (white bars) and extrapolated at time=∞ from fit in C (black bars). $k'_{S4}$ calculated from equation 1. *$P<0.05$ compared with wild-type.
the PIP₂ affinity (Kₐₑ₅⁡/Kₐₑ₅), and the voltage sensor integrity (kₐₑ₅⁡/kₐₑ₅). Such an approach prevents misinterpretation that may have been caused by allosteric effects of the mutations on the channel activity. An alternative method to measure more directly the channel affinity for PIP₂ would be a binding assay. However, such assays are usually made on segments of the channel (eg, the N or C termini). Truncation may modify dramatically the structure of the on segments of the channel (eg, the N or C termini). For example, a recent attempt to establish a PIP₂-binding assay was unable to reveal PIP₂ binding to Kᵥ₅₆ channel N or C termini, although PIP₂ interaction with several residues of the N and C termini was clearly demonstrated by mutagenesis.²,¹¹

A recent article²² suggests a downregulation by PIP₂ of IKᵣ (generated by KCNQ1–KCNE1) in guinea pig cardiac myocytes. This is apparently inconsistent with several studies, including ours,⁹,¹⁹ that suggest an activation of KCNQ1 channel by PIP₂. The study by Ding et al is also apparently inconsistent with the fact that the mutants studied here present a decreased PIP₂ affinity and provoke a loss-of-function phenotype in humans (long QT syndrome). One explanation to resolve this discrepancy is that in native tissues, direct PIP₂ activation of the channel may be present but masked by a stronger PIP₂ downregulation via as yet unidentified regulatory pathways. This hypothesis should be further tested in the future.

It was shown previously that intracellular application of polyvalent cations, including Mg²⁺, Ca²⁺, poly-l-lysine, and La³⁺, decreases the activity of PIP₂-regulated ion channels (Kᵥ₅₆, Kir2.1, and Human Ether-a-go-go–Related Gene) by screening the PIP₂-negative charges and preventing the phosphoinositide interaction with positive residues of the channel.⁶,¹²,²³ Similarly, KCNQ1 activity is decreased by poly-l-lysine, Ca²⁺, or Mg²⁺ application to the cytosolic face of the channel.⁹,¹⁹ Here we showed that Mg²⁺ is less potent to decrease the activity of mutant R539W because the equilibrium constant (kₑ₅⁡/kₑ₅) for R539W was significantly greater than for wild-type and other mutants measured in the presence of Mg²⁺ (Figure 7D). This observation suggests that the access of Mg²⁺ to PIP₂ is somehow impaired by the R539W mutation affecting the equilibrium constant.

The present work supports further similarities between inward rectifying channels and voltage-dependent potassium channels.⁹,²⁴ In both categories, an alteration in channel–PIP₂ interaction could lead to hereditary disorders: Andersen’s and Bartter’s syndromes for Kir1.1 and Kir2.1 and long QT syndrome for KCNQ1. In inward rectifier channels (2 transmembrane domains), the residues supporting PIP₂ interaction are located in the N terminus and the C terminus. In voltage-dependent Kv channels (6 transmembrane domains), the residues supporting PIP₂ interaction are likely located in the S₄–S₅ linker (R243) and the C terminus (R539 and R555). The S₄–S₅ linker in Kv channels may correspond to the N terminus of inward rectifying channels, whereas the C terminus in Kv channels may correspond to the C terminus in inward rectifiers. This comparison provides new insight on the functional relationship between the voltage sensor and the gate in Kv channels. Because the S₅–S₆ segment of Kv channels corresponds to the functional entity of an inward rectifier (M1–M2) with similar PIP₂ binding, and because PIP₂ binding controls the gate in inward rectifiers,²⁵ it is possible that the voltage sensor modulates PIP₂ binding of the channel gate (ie, that the PIP₂–channel interaction would link the voltage sensor and the gate). Our observation that R243H mutation affects PIP₂ binding and the voltage sensor supports this hypothesis.

The present study further confirms the physiological relevance of KCNQ1–PIP₂ interaction as suggested previously.⁹ Among the 100 KCNQ1 mutations reported so far, only a few have been linked to a dysfunction of KCNQ1 at the molecular level, including defective trafficking and a dominant-negative effect.²⁶ This study describes a novel molecular mechanism leading to the long QT syndrome and identifies its nature (electrostatic), the partner (a phospholipid), and the functional consequence of the altered interaction (a decrease in the channel open state).

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Impaired KCNQ1–KCNE1 and Phosphatidylinositol-4,5-Bisphosphate Interaction Underlies the Long QT Syndrome

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