Adult Ventricular Myocytes Segregate KCNQ1 and KCNE1 to Keep the $I_{\text{Ks}}$ Amplitude in Check Until When Larger $I_{\text{Ks}}$ Is Needed

Min Jiang, PhD; Yuhong Wang, PhD; Gea-Ny Tseng, PhD

Background—KCNQ1 and KCNE1 assemble to form the slow delayed rectifier ($I_{\text{Ks}}$) channel critical for shortening ventricular action potentials during high β-adrenergic tone. However, too much $I_{\text{Ks}}$ under basal conditions poses an arrhythmogenic risk. Our objective is to understand how adult ventricular myocytes regulate the $I_{\text{Ks}}$ amplitudes under basal conditions and in response to stress.

Methods and Results—We express fluorescently tagged KCNQ1 and KCNE1 in adult ventricular myocytes and follow their biogenesis and trafficking paths. We also study the distribution patterns of native KCNQ1 and KCNE1, and their relationship to $I_{\text{Ks}}$ amplitudes, in chronically stressed ventricular myocytes, and use COS-7 cell expression to probe the underlying mechanism. We show that KCNQ1 and KCNE1 are both translated in the perinuclear region but traffic by different routes, independent of each other, to their separate subcellular locations. KCNQ1 mainly resides in the jSR (junctional sarcoplasmic reticulum), whereas KCNE1 resides on the cell surface. Under basal conditions, only a small portion of KCNQ1 reaches the cell surface to support the $I_{\text{Ks}}$ function. However, in response to chronic stress, KCNQ1 traffics from jSR to the cell surface to boost the $I_{\text{Ks}}$ amplitude in a process depending on Ca binding to CaM (calmodulin).

Conclusions—in adult ventricular myocytes, KCNQ1 maintains a stable presence on the cell surface, whereas KCNE1 is dynamic in its localization. KCNQ1 is largely in an intracellular reservoir under basal conditions but can traffic to the cell surface and boost the $I_{\text{Ks}}$ amplitude in response to stress. (Circ Arrhythm Electrophysiol. 2017;10:e005084. DOI: 10.1161/CIRCEP.117.005084.)

Key Words: action potentials ■ calcium ■ calmodulin ■ potassium channels ■ sarcoplasmic reticulum

The slow delayed rectifier ($I_{\text{Ks}}$) channel is composed of pore-forming KCNQ1 channel and modulatory KCNE1 subunits. $I_{\text{Ks}}$ functions as “repolarization reserve” in ventricular myocytes. Under basal conditions, $I_{\text{Ks}}$ has small amplitudes and activates slowly. Other larger and faster-activating potassium currents are responsible for repolarizing ventricular action potentials. However, when β-adrenergic tone is high, $I_{\text{Ks}}$ becomes larger and activates faster. Enhanced $I_{\text{Ks}}$ is critical for shortening ventricular action potential duration in the face of tachycardia and increased L-type Ca current ($I_{\text{CaL}}$), allowing sufficient diastolic intervals for chamber filling. Loss-of-function mutations in KCNQ1 lead to long-QT syndrome type-1, which poses a high risk for ventricular fibrillation when β-adrenergic tone is high. On the other hand, gain-of-function mutations in KCNQ1 have been linked to arrhythmogenic short-QT syndrome type-2 and familial atrial fibrillation. These clinical observations highlight the importance of keeping the $I_{\text{Ks}}$ amplitude in check under basal conditions, while allowing upward adjustment when larger $I_{\text{Ks}}$ is needed.

$I_{\text{Ks}}$ remodeling in diseased hearts may contribute to acquired arrhythmia. $I_{\text{Ks}}$ is downregulated in canine models of subacute and chronic myocardial infarction, contributing to acquired long QT. On the other hand, $I_{\text{Ks}}$ is upregulated in a canine model of nonischemic cardiomyopathy and in atrial myocytes from patients with chronic atrial fibrillation. Whether diseased conditions may alter the subcellular distribution of $I_{\text{Ks}}$ components, and subsequently the $I_{\text{Ks}}$ amplitude, has not been investigated.

No lone KCNQ1 currents have been described for cardiac myocytes, and KCNE1 by itself does not have channel-forming capability. Therefore, KCNQ1 and KCNE1 are obligatory partners in forming the $I_{\text{Ks}}$ channels. A logic expectation is that KCNQ1 and KCNE1 should be well colocalized on myocyte surface to conduct $I_{\text{Ks}}$. However, our observations from guinea pig ventricular myocytes indicate that this is not the case: KCNQ1 exhibits a striation pattern along the z lines, whereas KCNE1 is on the peripheral surface. The mechanism for KCNQ1 and KCNE1 segregation and its implication for the control of $I_{\text{Ks}}$ amplitude in normal
Fluorescence In Situ Hybridization of mRNAs
We used QuantiGene ViewRNA ISH Cell Assay kit from Affymetrix to detect Q1-GFP and E1-dsR mRNAs along with the GFP and dsR protein fluorescence signals in single cardiac myocytes.

Confocal Microscopy
We used Zeiss 710 confocal microscope to image (immuno)fluorescence signals in fixed cells or to monitor Q1-GFP mobility in live cells by fluorescence recovery after photobleaching.

Statistical Analysis
Statistical analysis was done using SigmaStat v 2. Comparison between 2 groups was done using unpaired t test. Multiple-group comparison was done using 1-way ANOVA, and if significant difference was detected, followed by pairwise comparison with Tukey’s test. Absolute P values are reported unless P <0.001.

Supplemental Material
Expanded methods are presented in the Data Supplement.

Results
KCNQ1 and KCNE1 Are Largely Segregated in Canine Ventricular Myocytes
Canine ventricular myocytes express functional \( I_{Ks} \) (Figure 1A). Figure 1B shows that whereas KCNE1 is distributed on the peripheral surface, KCNQ1 exhibits a prominent striation pattern in the myocyte center. Selected cross-sectional views (XZ plane images reconstructed from \( z \) stacks) do show that the 2 overlap on the peripheral surface in a patchy manner (Figure 1B). Figure 1C shows that the KCNQ1 and KCNE1 distribution patterns in canine ventricular myocardium are very similar to those observed in isolated myocytes, ruling out the influence of cell isolation on KCNQ1 and KCNE1 distribution. We further use adenoviruses to express GFP-tagged KCNQ1 and dsRed-tagged KCNE1 (Q1-GFP and E1-dsR) in canine ventricular myocytes, to directly observe their distribution without using antibodies. Q1-GFP and E1-dsR have been validated in terms of their channel function and trafficking patterns.11 Figure 1D shows that their distribution patterns in canine ventricular myocytes, to directly observe their distribution without using antibodies. Q1-GFP and E1-dsR have been validated in terms of their channel function and trafficking patterns.11 Figure 1D shows that their distribution patterns in canine ventricular myocytes, to directly observe their distribution without using antibodies. Q1-GFP and E1-dsR have been validated in terms of their channel function and trafficking patterns.11
of native KCNQ1 and KCNE1 in guinea pig and canine ventricular myocytes, respectively.

How is the segregation between KCNQ1 and KCNE1 accomplished? We consider 2 scenarios. First, KCNQ1 and KCNE1 assemble into $I_{Ks}$ channels early during biogenesis as previously proposed and reach the cell surface together. After reaching the cell surface, the assemblies dissociate, with KCNE1 and a small portion of KCNQ1 staying on the surface while the majority of KCNQ1 enters a striation compartment. Second, newly translated KCNQ1 and KCNE1 traffic to their separate subcellular destinations, with only a portion of the 2 assembled into $I_{Ks}$ channels after they have reached the surface.

KCNQ1 and KCNE1 Are Segregated Early During Biogenesis

To differentiate between these 2 possibilities, we incubate canine ventricular myocytes with adenoviruses harboring Q1-GFP and E1-dsR for different durations (6–48 hours). At specified times, myocytes are fixed, and the distribution patterns of Q1-GFP and E1-dsR are examined. The beginning of adenovirus incubation serves to synchronize their translation. By fixing myocytes at different time points, we can deduce where the 2 originate and whether they travel together or separately. To avoid overexpressing Q1-GFP or E1-dsR, which may cause non-native trafficking patterns, we use $\approx 1 \times 10^7$ plaque-forming units per milliliter for Adv-Q1-GFP and $\approx 0.5 \times 10^7$ plaque-forming units per milliliter for Adv-E1-dsR during incubation. Figure S1 in the Data Supplement shows that, on average, Q1-GFP and E1-dsR are expressed at levels of 50% to 100% of their native counterparts in the myocytes.

We detect Q1-GFP and E1-dsR by indirect immunofluorescence, which facilitates target detection by amplifying the signals. This is particularly important during early time points when protein expression levels are low. Negative control (omitting primary antibodies, Figure 2A) and positive control (intrinsic fluorescence signals, Figure 2C) confirm the authenticity of the immunofluorescence signals. We carry out 7 independent experiments. The key observations are similar. For illustration purpose, we focus on 1 experiment (Figure 2B). Quantitative results are presented in Figure S2 in the Data Supplement.

The earliest time point when Q1-GFP and E1-dsR can consistently detected is 10 hours. Both appear in the perinuclear zone. For both, the perinuclear zone signals increase from 10 to 18 hours and then decline. Q1-GFP signals spread...
from the perinuclear zone to other cellular regions in a striation pattern along the z lines. Newly translated Q1-GFP is rarely detected in vesicular compartments. On the other hand, E1-dsR enters a prominent vesicular compartment that peaks at 18 hours and then declines. Q1-GFP and E1-dsR signals in the cell periphery gradually increase from 18 to 36 hours. The percentage of signals in cell periphery is higher for E1-dsR than Q1-GFP. A quasi-steady state is reached after 36 hours of incubation. Movies III through V in the Data Supplement provide 3-dimensional visualization of Q1-GFP and E1-dsR distribution patterns in canine myocytes that have been incubated with adenoviruses for 18, 24, and 36 hours. In some cases (e.g., the 18 hours myocyte in Movie III in the Data Supplement), Q1-GFP and E1-dsR originate from around different nuclei in the same myocyte. Furthermore, Q1-GFP and E1-dsR expressed separately exhibit the same distribution patterns as when they are coexpressed (Figure S3 in the Data Supplement). These data indicate the independent nature of biogenesis and trafficking of Q1-GFP and E1-dsR in adult ventricular myocytes.

E1-dsR Travels by the Conventional Secretory Pathway but Q1-GFP Does Not

To understand how newly translated Q1-GFP and E1-dsR travel in adult ventricular myocytes, we compare their distribution patterns with those of key organelles in the conventional, Golgi-dependent, secretory pathway. The organization of conventional secretory pathway has not been systematically examined in adult ventricular myocytes but has been well characterized for COS-7 cells. Figure 3A shows that protein translation sites (nuclear envelope and rough endoplasmic reticulum [rER]) can be marked by ER-resident proteins, calnexin and BiP (binding immunoglobulin protein). Newly translated proteins are transported from ER to Golgi through an intermediate compartment, marked by ERGIC-53. N-glycosylated proteins are processed during transit through the Golgi apparatus, becoming complex glycosylated and capable of binding wheat germ agglutinin (WGA). Golgi-processed proteins traffic to the plasma membrane in WGA-positive structures. Finally, βCOP (coat protein) marks COPII vesicles that mediate Golgi-to-ER and intra-Golgi transports.

Figure 3B shows that in adult ventricular myocytes, calnexin and BiP, now termed sarco/endoplasmic reticulum (SR/ER) markers, exhibit a striation pattern along the z lines, suggesting that jSR (junctional SR) may be active in protein translation/folding. ERGIC-53–positive vesicles are widely distributed in myocytes. There are large round structures aligned with the myocyte longitudinal axis positive for both WGA and βCOP, suggesting that these are the Golgi apparatus and post-Golgi structures.

Figure 3C shows that E1-dsR vesicles are positive for both WGA and βCOP, indicating that newly translated E1-dsR enters trans-Golgi network and post-Golgi structures, that is, it traverses the conventional secretory pathway. On the other hand, there is no overlap between Q1-GFP and WGA or
βCOP (Figure 3D), suggesting that newly translated Q1-GFP travels through a distinctly different pathway.18

Both Q1-GFP and E1-dsR Are Translated in the Perinuclear Zone

Is it possible that the distinctly different distribution patterns of newly translated Q1-GFP and E1-dsR reflect separate translation sites? To address these questions, we use fluorescence in situ hybridization to detect Q1-GFP and E1-dsR mRNAs (by red and green fluorophore-conjugated probes, respectively) and their protein products (by intrinsic fluorescence) in the same myocyte. To avoid conflicts in fluorescence signals, we express Q1-GFP and E1-dsR separately. These experiments are conducted on adult ventricular myocytes from young SHRs (SYs), which express functional IKs. Figure S4 in the Data Supplement shows that the distribution patterns of newly translated Q1-GFP and E1-dsR in SHR myocytes are similar to those in canine myocytes. Importantly, Q1-GFP and E1-dsR expressed separately exhibit the same distribution patterns as when they are coexpressed in SHR myocytes.

Figure 4A shows that Q1-GFP mRNA clusters around nuclei in the space between nuclei and the cell membrane. Q1-GFP protein is detected in the perinuclear zone and in regions away from the nuclei where no Q1-GFP mRNA signals are detected. Movie VI in the Data Supplement depicts 3-dimensional visualization of Q1-GFP mRNA and protein in this myocyte. Similar observations are obtained from 13 myocytes. These data indicate that Q1-GFP is translated in the perinuclear zone and spreads to other cellular regions in a striation pattern.

E1-dsR mRNA also clusters around nuclei in the space between nuclei and cell membrane (Figure 4B). E1-dsR vesicles are detected in regions where no E1-dsR mRNA is detected (Movie VII in the Data Supplement). Similar observations are obtained from 11 myocytes. These observations
indicate that E1-dsR is translated in the perinuclear zone and spreads to other cellular regions in vesicles.

The Striation Pattern of KCNQ1 Represents Localization in JSR

The striation pattern of native KCNQ1 and Q1-GFP in adult ventricular myocytes may represent transverse (t)-tubule or jSR localization. Distinction between the 2 has important implications: KCNQ1 channels in the t-tubule may contribute to surface electric activity, whereas KCNQ1 in jSR likely serves as an intracellular reservoir. Figure 5A shows that KCNQ1 maintains its striation pattern in myocytes where t-tubules are missing. On the other hand, Figure 5B shows that the striation pattern of native KCNQ1 coincides with those of SR proteins: RyR2 (ryanodine receptor type 2), JPH-2 (junctophilin-2), SERCA2a (SR/ER Ca pump), CSQ2 (calsequestrin 2), and calnexin. The distance between t-tubule and jSR membranes is only 10 to 25 nm, beyond the detection limit of confocal and even high-resolution microscopy. Therefore, we use 2 alternative approaches to probe the subcellular environments of Q1-GFP/native KCNQ1 and E1-dsR/native KCNE1 in adult ventricular myocytes.

First, we immunoprecipitate Q1-GFP and E1-dsR along with their binding partners from myocytes. This allows us to deduce their immediate subcellular environment based on their binding partners. The top 2 rows of Figure 5C confirm the effectiveness of our coimmunoprecipitation approach: GFP-Nb pulls down E1-dsR associated with Q1-GFP, whereas dsR rabbit pAb pulls down Q1-GFP associated with E1-dsR. The middle 5 rows of Figure 5C show that Q1-GFP can be coimmunoprecipitated with SR/ER markers: RyR2, SERCA2a, CSQ2, calnexin, and JPH-2. Importantly, CSQ2 is an SR/ER luminal protein. The fact that CSQ2 can be coimmunoprecipitated with Q1-GFP indicates that Q1-GFP is embedded in the SR/ER membrane with its extracellular domain facing the SR/ER lumen accessible to CSQ2. On the other hand, E1-dsR cannot be coimmunoprecipitated with RyR2, CSQ2, or JPH-2. E1-dsR can be coimmunoprecipitated with calnexin, reflecting nascent E1-dsR being translated in the rough ER. We also detect very faint SERCA2a signal in the dsR pAb immunoprecipitate, at a level much weaker than SERCA2a signal in the GFP-Nb immunoprecipitate. The remaining panels of Figure 5C will be discussed later (Discussion).
The second approach is to separate the SR/ER and sarcolemmal (SL) compartments and quantify their contents of native KCNQ1 and KCNE1. These experiments are conducted on guinea pig hearts using a previously described protocol.13 Membrane vesicles prepared from whole heart homogenates are subjected to Ca oxalate loading through the membrane Ca pump activity. Because the SR/ER vesicles express a higher Ca pump activity than SL vesicles, a brief loading duration (5 minutes at 36°C) will preferentially load the SR/ER vesicles with Ca oxalate, making them heavier than the SL vesicles. This allows separation between the 2 by ultracentrifugation through sucrose gradients. The resulting SL and SR gradients are noted on top of Figure 5D, based on the distribution patterns of SR/ER markers (RyR2, SERCA2a, and calnexin) and SL markers (NCX*, glycosylated Na/Ca exchanger, and Na–K pump). The distribution of native KCNQ1 follows the SR/ER markers, whereas the distribution of native KCNE1 follows the SL markers. Figure 5C and 5D together provide direct evidence that KCNQ1 (as Q1-GFP or native counterpart) resides in the SR/ER compartment, but KCNE1 is mainly on the cell surface.

KCNQ1 Can Move Among Cellular Areas Within the SR/ER Compartment

In interphase cells, the ER is a single organelle with interconnecting membranes throughout the cell.17 If Q1-GFP is in the SR/ER compartment of adult ventricular myocytes, it may be able to move from one region to another through the SR/ER membranes. We test this possibility using the technique of “fluorescence recovery after photobleaching”. We bleach a small area in the center of canine ventricular myocyte where Q1-GFP is in clear striations. Q1-GFP can move from unbleached areas to replenish the fluorescence in the bleached area, and the recovered fluorescence resumes the original striation pattern (n=41; Figure 6Aa; Movie VIII in the Data Supplement). These data support the above scenario: KCNQ1 resides in the SR/ER membranes. The striation pattern reflects its high abundance in the jSR regions.
The mobility of Q1-GFP in ventricular myocytes is much slower than that in the ER compartment of COS-7 cells (Figure 6Ac). Protein mobilities are influenced by their sizes: large, multiple-protein complexes move slower than small protein complexes. Figure 5C reveals that Q1-GFP can interact with multiple SR/ER proteins, possibly forming one or more forms of macromolecular complexes. This may slow its mobility in ventricular myocytes. To test this possibility, we coexpress JPH-2, one of the strongest Q1-GFP–binding partners, in COS-7 cells and analyze its effect on Q1-GFP mobility. Figure 6Ba and 6Bb confirm that JPH-2 can be coimmunoprecipitated with Q1-GFP, and JPH-2 puncta (representing ER–plasma membrane junctions) overlap with Q1-GFP in the ER. JPH-2 coexpression significantly slows Q1-GFP mobility in the ER compartment (prolonging the time constant of fluorescence recovery, \( \tau_{\text{recovery}} \), although no change in the percent of steady-state Q1-GFP recovery, % mobile, Figure 6Bc through 6Be).

These data support the notion that in ventricular myocytes, KCNQ1 is stabilized by extensive interactions with SR/ER-resident proteins. On the other hand, cell peripheral Q1-GFP recovers at a significantly faster rate than cell center, SR/ER-resident Q1-GFP (Figure 6Ab and 6Ac). This difference suggests that cell peripheral Q1-GFP forms smaller molecular complexes than its counterpart in the SR/ER compartment.

KCNQ1 Reservoir in the SR/ER Compartment Endows Adult Ventricular Myocytes With the Ability to Increase \( I_{\text{Ks}} \) Amplitude in Times of Need

Can KCNQ1 move from the intracellular SR/ER reservoir to cell surface and boost the \( I_{\text{Ks}} \) amplitude when a larger \( I_{\text{Ks}} \) is needed? Data presented in Figure 7 support such a scenario. SHR ventricular myocytes express native KCNQ1, KCNE1, and functional \( I_{\text{Ks}} \) channels, whose currents can be separated from other much larger currents, such as transient outward current \( I_{\text{to}} \), as HMR1556-sensitive currents. Old SHRs (22–24 months of age) exhibit symptoms of heart failure: severe interstitial fibrosis, left atrial enlargement, and thrombosis (indicative of atrial fibrillation; Figure 7A), as well as ventricular wall thinning and chamber dilatation. The \( I_{\text{to}} \) current density in left ventricular epicardial myocytes from SO is markedly reduced than those from young SHRs (4–6 months of age; data not shown). However, the \( I_{\text{Ks}} \) current density is larger in left ventricular epicardial myocytes from SO than SY, although the gating kinetics is not altered (eg, no shift in the voltage dependence of \( I_{\text{Ks}} \) activation, Figure 7B). Immunoblot experiments on whole tissue lysates from left ventricular epicardial samples show that the KCNQ1 protein level is only modestly elevated in SO than SY, but KCNE1 protein level is reduced in SO than SY, especially in severely hypertrophied hearts (Figure 7C). Therefore, the changes in KCNQ1 and
KCNE1 protein levels cannot explain the marked increase in $I_{Ks}$ current density in SO myocytes. We monitor the distribution patterns of native KCNQ1 and KCNE1 in the SHR myocytes (Figure 7D). Whereas KCNE1 is stable on the peripheral surface of both SY and SO myocytes, the patterns of KCNQ1 are dramatically different. In SY myocytes, KCNQ1 exhibits clear striations along the $z$ lines, the same as KCNQ1 in canine and guinea pig myocytes (Figure 1), indicating jSR localization. In SO myocytes, KCNQ1 disappears from the jSR compartment but clusters to the peripheral surface. As a result, the degree of KCNQ1/KCNE1 colocalization in myocyte periphery is significantly higher in SO than SY myocytes (Figure 7E).

The above data are not uniquely SHR phenomenon. Figure S5 in the Data Supplement illustrates that the increase in $I_{Ks}$ amplitude in chronically stressed canine ventricular myocytes (cardiomyopathy induced by chronic premature ventricular contractions) is accompanied by a similar shift of KCNQ1 from the jSR to the SL compartment.

**Persistent AT1R Stimulation Promotes KCNQ1 Trafficking to Cell Surface in a Ca- and Calmodulin-Dependent Manner**

What may trigger KCNQ1 to move from jSR to cell surface? It has been shown that in SO heart local angiotensin II (Ang II) levels are elevated, leading to a persistent activation of AT1R and its downstream signaling cascades. Chronic ventricular pacing in the canine heart also causes local Ang II elevations and AT1R activation. Therefore, we test the effect of persistent AT1R stimulation on $I_{Ks}$ channel function and KCNQ1/KCNE1 distribution. The experiments are conducted in COS-7 cells coexpressing AT1R and recombinant $I_{Ks}$ (KCNQ1 plus KCNE1). Ang II pretreatment causes an increase in the $I_{Ks}$ density (Figure 8Aa) and a negative shift in the threshold voltage of $I_{Ks}$ activation (Figure 8Ab). The latter change can be explained by an increase in cell surface KCNQ1 without a concomitant increase in cell surface KCNE1. Indeed, KCNQ1 expressed alone responds to Ang II pretreatment with a prominent increase in current density without shifting the threshold of activation (Figure 8Ac). Biotinylation experiments confirm that Ang II pretreatment increases cell surface KCNQ1 but not KCNE1 (Figure 8B, left panel).

AT1R stimulation can activate phospholipase C, which hydrolyzes membrane PIP$_2$ (phosphatidylinositol 4,5-bisphosphate) producing IP$_3$ (inositol 1,4,5-trisphosphate). IP$_3$ induces Ca release through IP$_3$ receptors in the SR/ER membrane, leading to $[Ca^{2+}]_{i}$ elevation. It has been shown that $[Ca^{2+}]_{i}$ elevation can increase native $I_{Ks}$ amplitude in guinea pig ventricular myocytes in a CaM (calmodulin)-dependent manner. However, in the case of KCNQ1/KCNE1, the downstream effects of CaM dependence are not clear.
This occurs through Ca binding to CaM constitutively associated with the C terminus of KCNQ1. To test whether this signaling cascade is involved in the increase in cell surface KCNQ1, we test the effects of 2 interventions on the \( I_{Ks} \) response to AT1R stimulation: (1) preventing Ca binding to CaM, by displacing the native CaM with a Ca-binding–deficient CaM mutant, CaM1234, through overexpression, and (2) inhibiting Ca release through IP3 receptor, by including 5 \( \mu \text{mol/L} \) 2-aminoethoxydiphenyl borate during Ang II incubation. Figure 8Ac, right panels, show that both CaM1234 overexpression and IP3 receptor inhibition prevent AT1R stimulation from increasing \( I_{Ks} \) current density. Biotinylation experiments show that AT1R stimulation fails to increase cell surface KCNQ1 under these 2 conditions (Figure 8B, right panels).

**Discussion**

The following are our major findings. First, there is a general \( I_{Ks} \) property in adult ventricular myocytes: most KCNQ1 is in an intracellular jSR reservoir revealed in microscopic experiments as striations along the \( z \) lines, whereas KCNE1 is on the peripheral surface. Second, the segregation of KCNQ1 and KCNE1 begins during their biogenesis. Although both proteins are translated in the perinuclear zone, newly translated KCNQ1 and KCNE1 traffic by different routes, independent of each other, to their separate destinations. Third, KCNE1 traffics in vesicles through the conventional, Golgi-dependent pathway to reach the myocyte surface. On the other hand, fluorescence recovery after photobleaching experiments show that KCNQ1 can move within the SR/ER compartment in cardiac myocytes. Fourth, because of the segregation between KCNQ1 and KCNE1, under basal conditions, only a small portion of KCNQ1 is on the myocyte surface, assembled with KCNE1 to support the basal \( I_{Ks} \) channel function. However, chronic stress (in SO and canine model of chronic premature ventricular contractions) promotes KCNQ1 exit from the SR/ER compartment. KCNQ1 moves to the myocyte surface and assembles with KCNE1 to boost the \( I_{Ks} \) amplitude. Fifth, experiments in the COS-7 expression system suggest that the above KCNQ1 trafficking process depends on Ca binding to CaM.

**What Stabilizes KCNE1 on the Myocyte Surface?**

When expressed in COS-7 cells, cell surface KCNE1 is not stable but experiences rapid endocytosis. To explore the mechanism stabilizing KCNE1 on the myocyte surface, we test whether KCNE1 interacts with scaffolding proteins known, or potentially able, to anchor KCNE1. Telethonin and FHL-2 (four and half LIM-domain containing protein 2) have been suggested to anchor KCNE1 in cardiac myocytes.\(^{27,28}\)
channels cluster to caveolae in guinea pig ventricular myocytes that are stabilized by caveolin-3. Finally, α-actinin may stabilize surface proteins indirectly. We cannot detect any of these proteins in dsR pAb immunoprecipitate from myocyte lysates expressing E1-dsR. Further experimentation with a global search strategy is required to answer this question.

Implications for the Regulation of \( I_{\text{Ks}} \) Amplitude in Adult Ventricular Myocytes

Ca binding to KCNQ1-associated CaM leads to an increase in the number of \( I_{\text{Ks}} \) channels on the cell surface.^{22,23} Here, we show that in adult ventricular myocytes, most KCNQ1 is in the jSR with its cytoplasmic C-terminal domain facing the dyadic space. KCNE1 is on the cell surface, ready to form \( I_{\text{Ks}} \) channels with its cytoplasmic C-terminal domain facing the dyadic space. KCNE1 is on the cell surface, ready to form \( I_{\text{Ks}} \) channels when KCNQ1 is available. We suggest that the cyclic elevations of [Ca\(^{++}\)] in the dyadic space during cardiac action potentials serve as a trigger for KCNQ1 to exit the jSR and reach the cell surface, where it assembles with KCNE1 to support the \( I_{\text{Ks}} \) function.

The most important physiological function of \( I_{\text{Ks}} \) is to respond to β-adrenergic stimulation: \( I_{\text{Ks}} \) becomes larger and activates faster, so that it can help shorten the ventricular action potentials. β-adrenergic stimulation increases \( I_{\text{Ks}} \) by 2 known mechanisms: (1) accumulating \( I_{\text{Ks}} \) channels in activated state during β-adrenergic–induced tachycardia (operating in seconds),^{10,11} and (2) protein kinase A–mediated phosphorylation of \( I_{\text{Ks}} \) components (operating in minutes).^{31} Live cell imaging shows that acute exposure of quiescent canine ventricular myocytes expressing Q1-GFP to β-adrenergic agonist (isoproterenol 1 µmol/L, 37°C, 30 minutes) does not induce trafficking events. However, in ventricular myocytes in situ, β-adrenergic stimulation can amplify [Ca\(^{++}\)] spike in the dyadic space during excitation–contraction coupling. This can promote KCNQ1 trafficking to the cell surface, constituting a third mechanism for β-adrenergic–induced increase in \( I_{\text{Ks}} \) that operates on the minutes-to-hour time scale.

Acute AT1R stimulation in canine and guinea pig ventricular myocytes induces a small and transient \( I_{\text{Ks}} \) enhancement followed by a steady decline, which likely reflectsPIP\(^{2-}\) depletion after phospholipase C activation. COS-7 expression of these proteins in dsR pAb immunoprecipitate from myocyte lysates expressing E1-dsR. Further experimentation with a global search strategy is required to answer this question.

Novelty of This Study

Our study provides the following novel insights into how adult ventricular myocytes regulate their \( I_{\text{Ks}} \) current amplitude to suit their needs: (1) KCNQ1 and KCNE1 are translated separately and transported in different routes, assembled into \( I_{\text{Ks}} \) after reaching the cell surface. This is different from the previous view that KCNQ1 and KCNE1 assemble into \( I_{\text{Ks}} \) early during biogenesis (in the ER or soon thereafter).^{14,15} (2) The striation pattern of KCNQ1 in adult ventricular myocytes reflects its localization in the jSR compartment. This is different from the previous belief that KCNQ1 is localized in the t-tubule membrane. (3) Chronic stress as seen in old SHR and premature ventricular contractions dog can promote KCNQ1 translocation from jSR to cell surface, leading to an increase in \( I_{\text{Ks}} \) in ventricular myocytes. This is different from the prevailing view that \( I_{\text{Ks}} \) is downregulated in diseased heart. However, we note that \( I_{\text{Ks}} \) indeed is downregulated in ventricular myocytes surviving myocardial infarction.^{7,8}

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Disclosures

None.

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A. Expanded methods

1. Molecular constructs
   KCNE1-dsR (E1-dsR) was created by fusing mono-dsRed to the C-terminus of stop-codon-removed KCNE1. KCNQ1-GFP (Q1-GFP) was a gift from Dr. Andrew Tinker (University College London). It is the 676-aa KCNQ1 isoform with eGFP fused to the C-terminus. E1-dsR and Q1-GFP were subcloned into a shuttle vector, Dual-CCM(-), for adenovirus production (Vector Biosystems, Malvern, PA). Angiotensin type 1 receptor (AT1R) was a gift from Dr. Yanfang Xu (Hebei Medical University, Shijiazhuang, China). A Ca-binding deficient calmodulin mutant (CaM123a) was a gift from Dr. Geoffrey S. Pitt (Weil Cornell Medical College). The plasmid pOPINE encoding GFP nanobody (GFP Nb) was a gift from Brett Collins (AddGene plasmid#49172).

2. Animal experiments
   Three animal species were used: (1) canine: strain – mongrel, number used – 7, (2) rat: strain – spontaneously hypertensive (SHR), number used - 9, and (3) guinea pig: strain – Harley, number used – 16. Animal procedures were in accordance with institutional guidelines (VCU IACUC protocol # AM10294).

Ventricular myocyte preparation: isolation, culture and adenovirus incubation
   Canine and SHR ventricular myocytes were isolated as described previously. Briefly, the left circumflex coronary artery perfusing a wedge of left ventricular (LV) free wall of canine heart or the aorta of SHR heart was cannulated and mounted on a Langendorff apparatus. The tissue was perfused with the following solutions sequentially (all at 36°C, oxygenated): normal Tyrode’s (mmol/L: NaCl 146, MgCl₂ 0.5, HEPES 5, dextrose 5.5, KCl 4, CaCl₂ 2, pH 7.4) for 5 min, nominally Ca-free Tyrode’s with 1% fat-free bovine serum albumin (BSA) for 5 min, and the same Ca-free, BSA-containing solution with collagenase (type II, Worthington, 1 mg/ml), for 20-30 min or till the tissue became soft. For canine LV wedge, the epicardium and endocardium were removed, and the midmyocardial tissue was minced in Ca-free, BSA-containing Tyrode’s followed by gentle trituration to release single myocytes. For SHR heart, the atria were removed, and the ventricular tissue was minced/triturated as described above.

   To set up myocyte culture and adenovirus infection, myocytes were allowed to recover in Ca-free, BSA-containing Tyrode’s solution for ~ 1 hr, at room temperature. Then [Ca] in the solution was gradually raised to 1 mM, over a period of ~ 2 hrs. The myocytes were then plated on mouse laminin- or matrigel- coated coverslips, and incubated in medium 199 (mmol/L: NaHCO₃ 26, HEPES 25, dextrose 5.6, acetate 0.6, and amino acids, with the following supplements: carnitine 5, creatine 5, taurine 5, BSA, penicillin/streptomycin and 2% FCS) in 36°C moist 5% CO₂ incubator. After culturing for 4-6 hr, myocytes were gently rinsed with fresh medium 199 to remove floating (mostly dead) myocytes. Adv-Q1-GFP and/or Adv-E1-dsR were added to the myocytes (at 1×10⁷ and 0.5×10⁷ plaque-forming units ‘PFU’ per ml, respectively), with gentle swirling to facilitate contacts between myocytes and viral particles. Myocytes in adenovirus-containing medium were returned to 36°C incubator and this was time zero of adenovirus-incubation time.

Ventricular myocardium experiments: cryosections and (immuno)histochemistry
   Canine left ventricular free wall (approximately 1x1x0.5 cm) was fixed by immersion in 4% paraformaldehyde at 4°C overnight. SY and SO hearts were fixed by perfusing the whole heart through aorta with 4% paraformaldehyde. After removing the atria, the ventricles were immersed in 4% paraformaldehyde at 4°C overnight. The samples were transferred to 40%
(w/v) sucrose in PBS and stored at 4°C until they settled to the bottom. The 40% sucrose saturated samples were embedded in OCT compound and slowly frozen in liquid nitrogen vapor. The frozen samples in OCT compound were stored at -70°C until experiments. Cryosections of 12-15 um thickness were cut using a cryostat, deposited on glass slides, and stored at -70°C until immunohistochemistry or histochemistry experiments.

Separation of sarcoplasmic reticulum (SR) and sarcolemma (SL) vesicles from guinea pig hearts

The method was modified from a published protocol⁹. Briefly, three guinea pig hearts were combined for one experiment. The hearts were homogenized in 15 ml of 10 mM NaHCO₃ (pH 7.0) with protease inhibitors. The homogenate was centrifuged at 14,000x g twice to remove debris. The supernatant was centrifuged at 45,000x g for 30 min to pellet vesicles. The vesicles were resuspended in 12 ml of 0.6 M KCl plus 30 mM imidazole, pH 7.0, and incubated on ice for 1 hr (to extract contractile proteins), followed by centrifugation at 45,000x g for 30 min. The purified vesicles were resuspended in 8 ml of the following solution (in mmol/L): imidazole 50, CaCl₂ 15, Tris-base 16, EGTA 16, KCl 100, pH 6.8. To initiate Ca-oxalate loading, 2 ml of ATP-Mg stock solution (mmol/L: ATP-Mg 150, Tris-base 150, pH 6.8) was added (final [ATP] 30 mmol/L), and the mixture was incubated at 37°C for 5 min. Then 0.5 ml of oxalate stock (mmol/L: K-oxalate 100, Tris-HCl 100, pH 6.8) was added (final [oxalate] 5 mmol/L) and the mixture was incubated at 37°C for 5 min. Pilot experiments showed that the ‘5 min duration’ of both steps was critical: prolonging the incubation time would start Ca-oxalate loading into SL vesicles and interfere with separation of SR from SL vesicles. The mixture was centrifuged at 105,000x g for 30 min to pellet the vesicles. To separate the heavier SR vesicles from the lighter SL vesicles, the pellet was resuspended in 2 ml of a high salt solution (mmol/L: KCl 200, Na-pyrophosphate 50, Tris-base 100, pH 7.2) plus 0.25 M sucrose. This solution was layered over a discontinuous sucrose gradient of 0.6, 0.8, 1.0 and 1.5 M sucrose in the same high salt solution, and centrifuged at 132,000x g for 2 hrs. Five fractions were collected: four interfaces (between 0.25 and 0.6 M, 0.6 and 0.8 M, 0.8 and 1 M, 1 and 1.5 M sucrose gradients), and the pellet at the bottom (in 1.5 M sucrose). All 5 fractions were solubilized in 1% Triton X-100 on ice for 1 hr, and after quantifying protein concentrations with a Micro BCA protein assay kit (Pierce), subjected to immunoblot analysis.

3. COS-7 experiments

COS-7 cells were cultured in DMEM (supplemented with 10% FCS, non-essential amino acids, and penicillin/streptomycin) in 36°C, 5% CO₂, moist incubator. COS-7 cells were transiently transfected with plasmids using lipofectamine2000 according to the manufacturer’s instruction.

To study the effects of persistent AT1R stimulation on the current amplitude and cell surface expression of KCNQ1 and KCNE1, COS-7 cells were transfected with cDNAs encoding AT1R, KCNQ1, without or with KCNE1, and in the latter case without or with cDNA encoding a Ca-binding deficient calmodulin mutant CaM₁₂₃₄⁴. The cDNA molar ratios of AT1R:KCNQ1:KCNE1:CaM₁₂₃₄ were 1:1:1: (0 or 1): (0 or 1). These 3 groups of cells are labeled as ‘Q1 alone’, ‘Q1/E1’, and ‘Q1/E1+CaM₁₂₃₄’ in Fig. 8. A small amount of CD8 cDNA was also included (48 ng/dish) to mediate low level expression of cell surface CD8 antigen. Before patch clamp recording, cells were incubated with CD8 mAb coated beads. Bead-decorated (i.e. transfected) cells were chosen for patch clamp. After culture for 24 hr, each group of cells was divided into 2 aliquots. One was incubated with angiotensin II (Ang II, 1 uM, 1 hr at 36°C, labeled as ‘+Ang II”), and the other was incubated under the same conditions without Ang II (-Ang II). An additional group of Q1/E1 cells was further treated with 2-aminoethoxydiphenyl borate (2APB, 5 uM) during ‘+Ang II’ or ‘-Ang II’ incubation. They are labeled as ‘Q1/E1+2APB’ in Fig. 8. At this concentration, 2APB was a selective IP₃ receptor antagonist. After incubation,
cells were patch clamped to record whole cell currents (perforated mode, room temperature), or subjected to biotinylation followed by whole cell lysis and immunoblot analysis, all in the absence of Ang II. During patch clamp recording, to avoid interference from time-dependent changes in channel expression and from the waning of Ang II effects, we alternated recordings from `-Ang II` and `+Ang II` cells and each batch of cells was patched for ~ 1 hr.

4. Confocal experiments

All imaging experiments were done using Zeiss 710 confocal microscope with 40x (myocytes) or 63x (COS-7) objective. The excitation/detection filter settings were (in nm): DAPI (405/415-587), Alexa-488 or eGFP (488/494-572), Alexa-568 or dsRed (561/572- 649) and Alexa 647 (633/642-709). Multi-color fluorescence detection was done in the sequential imaging mode. Z-stack images were obtained at the optimal optic thickness recommended by the ZEN program based on Nyquist theorem.

**Imaging fixed cells**

Fixed and membrane permeabilized cardiac myocytes or COS-7 cells attached to glass coverslips were incubated with primary antibodies diluted with blocking buffer to suitable concentrations at room temperature for 2 hr. After rinsing with PBS, cells were incubated with Alexa-conjugated secondary antibodies diluted 250 fold with blocking buffer at room temperature for 1 hr in dim light. After further incubation with DAPI (to label nuclei), and where indicated, with Alexa-conjugated wheat germ agglutinin (WGA, to label cell membrane including t-tubules, trans Golgi network and post-Golgi structures), cells were rinsed with PBS and the coverslips were mounted on glass slides with ProLong Diamond antifade reagent (Invitrogen). To quantify the degree of colocalization between native KCNQ1 and KCNE1 in SHR myocyte periphery, we used the program 'Volocity' to calculate the thresholded Pearson correlation coefficient between KCNQ1 and KCNE1 immunofluorescence signals in an area within 2 um from the cell boundary (defined as cell periphery) in the central XY plane of Z-stack. Threshold values were determined by pixel values in cell-free area.

To quantify Q1-GFP and E1-dsR fluorescence signals in perinuclear zone or cell periphery of canine ventricular myocytes that had been incubated with Adv-Q1-GFP and Adv-E1-dsR for different durations, the z-stack images of Q1-GFP, E1-dsR and WGA were exported from ZEN as original data in TIFF format. ImageJ imported these stack images, and created Z-projections of sum of fluorescence intensity. This step collapsed the 3D images into 2D. Then using the WGA image as a guide, the following regions of interest (ROIs) were selected: total contour of the cell, inner contour (~ 2 um within the cell contour), perinuclear zone and cell free area. The pixel values and areas of all ROIs were exported to Excel. Excel was used to subtract background (from cell free area), and calculate pixel contents (pixel values times areas) in ROIs. These numbers were used to calculate % of pixel content in the cell periphery (total – inner contour) and the perinuclear zone.

To quantify cellular area occupied by E1-dsR vesicles, the z-stack images of E1-dsR were exported from ZEN as original data in TIFF format. ImageJ imported these stack images, and created Z-projections of maximum of fluorescence intensity. This step collapsed the 3D images into 2D. Then the 2D image was thresholded, so that only vesicles were visible. We used an ImageJ function, *Particle Analysis*, to calculate % cell area occupied by E1-dsR vesicles.

**Imaging live cells**

All live cell imaging experiments were conducted at 37°C, in phenol-free HEPES-buffered medium or in normal Tyrode’s solution. To perform Q1-GFP fluorescence recovery after photobleaching (FRAP), the following regions of interest (ROIs) were specified: bleached area(s), neighborhood (area adjacent to the bleached area), reference (far from the bleached
area in the same cell or from another cell in the same field) and background (in cell-free area). Q1-GFP signal was detected at a low laser power (1% or lower, to avoid unintended photobleaching) once every 10 or 15 s. After the pre-bleach data were obtained, the intended bleached area(s) was scanned by 100% laser power repeated 10 or more times till the fluorescence was reduced to ~30% of pre-bleach level.

The changes in Q1-GFP fluorescence signals in all ROIs were monitored up to 25 min (canine ventricular myocytes) or 6-10 min (COS-7 cells). The mean pixel values and areas of all ROIs were exported to Microsoft Excel for data analysis. After subtracting the background mean pixel value, the pixel contents in other ROIs (mean pixel value x area) were normalized by respective pre-bleach values. In all experiments reported here, the degree of unintended bleaching, as was detected in the reference ROI, was 10-15% at the end of the observation time. In COS-7 cells, the recovery phase of normalized pixel content was fit with a single-exponential function: F(t) = F(0)+F(∞)[1-exp(-t/t_{recovery})], where F(0), F(t), and F(∞) were pixel contents at time ‘zero’, time ‘t’ after photobleaching, and at steady-state of recovery, and t_{recovery} was time constant of fluorescence recovery. The percent of Q1-GFP that was mobile (% mobile) was calculated as (F(∞)-F(0))/[1-F(0)])*100%. In canine ventricular myocytes the time course of fluorescence recovery required two-exponential fit. We used the fraction recovered 10 min after photobleaching for quantification and comparison.

5. Biochemical experiments

GPF Nb production and antibody conjugation to agarose beads

Production of GFP Nb (with a His_6 tag at the C-terminus) was based on a published protocol. Briefly, E coli strain BL21(DE3) was transformed with GFP Nb plasmid, and protein expression was induced by 1 mM IPTG for 20 hr at room temperature. The bacteria was harvested and sonicated on ice. After centrifugation, the supernatant (cleared lysate) was used for purification with Ni-NTA agarose kit (Qiagen) following the manufacturer’s instruction. GFP Nb and dsR rabbit pAb were conjugated to agarose beads using a Co-immunoprecipitation kit (Pierce) according to manufacturer’s instruction.

Coimmunoprecipitation (co-IP) experiments

Myocytes expressing Q1-GFP and E1-dsR were collected in buffer (mmol/L: NaCl 145, MgCl₂ 0.1, HEPES 15, EGTA 10, pH 7.4, with protease inhibitor cocktail) and sonicated. The homogenate was centrifuged to remove debris, solubilized in 0.5 % Triton X-100 for 1 hr on ice, and centrifuged to remove insoluble particles. The resulting whole cell lysate (WCL) was precleared by rocking with control agarose beads (without antibody) overnight at 4°C. Antibody (Ab)-conjugated beads were rocked in 1% BSA overnight at 4°C to block non-specific binding. The Ab-conjugated beads were added to the precleared WCL and rocked overnight at 4°C. Both the preclearing control beads and the WCL-incubated Ab-conjugated beads were washed extensively, and incubated with equal volume of 2x SDS sample buffer with mercaptoethanol at room temperature for 30 min to elute proteins bound to the beads. For each co-IP experiment we used immunoblot to analyze the following 4 samples: original WCL (positive control), eluent from the preclearing control agarose beads (negative control), supernatant (WCLs after incubation with Ab-conjugated beads, loading volume the same as the original WCL), and eluent from the Ab-conjugated beads (immunoprecipitate).

Biotinylation experiments

Live COS-7 cells were incubated with 1 mM amine-reactive biotin derivative (EZ-link-Sulfo-NHS-SS-biotin) on ice for 20 min. After quenching biotinylation with 100 mmol/L Tris-HCl (pH 7.5), cells were scraped into cold PBS plus protease inhibitor cocktail, and solubilized in 1% Triton X-100, on ice for 1 hr. A small fraction (2%) of the whole cell lysate (WCL) was saved for
immunoblots. The remaining WCL was incubated with Neutravidin-conjugated agarose beads by rocking at 4°C overnight. Biotinylated proteins bound to Neutravidin were eluted by incubating the beads with mercaptoethanol-containing 2x SDS sample buffer.

The WCL and biotinylated fraction from the same batch of cells were loaded side-by-side for SDS-polyacrylamide gel electrophoresis, and densitometry of immunoblot was used to quantify the band intensities in WCL and biotinylated lanes. The % of whole cell protein present on the cell surface was calculated as: % biotinylated = [b/(b+Wx49)]x100%, where ‘b’ and ‘W’ are band intensities of the biotinylated and whole cell lysate lanes, and ‘49’ reflects the fact that these lanes were loaded with ‘98% of WCL-equivalent’ and ‘2% of WCL’, respectively.

**Immunoblot experiments**

Samples were incubated with 2x SDS sample buffer plus mercaptoethanol at room temperature for 30 min, and loaded onto polyacrylamide gels of suitable concentrations (6 – 12%, depending on the sizes of proteins of interest). After electrophoresis, proteins were blotted onto PVDF membranes and probed with suitable antibodies. Immunoreactivity was visualized using SuperSignal West Femto Maximum Sensitivity ECL kit (Pierce), and densitometry quantification was done using FluorChem E (ProteinSimple).

**6. Fluorescence in situ hybridization ‘FISH’ experiments**

FISH experiments were conducted on ventricular myocytes from young SHR animals (2-4 months old, SY). Myocytes were plated on matrigel-coated glass coverslips, cultured and infected with Adv-Q1-GFP or Adv-E1-dsR as described above. At specified times, myocytes were fixed and stored in 4% PFA at 4°C until experiments (≤ 2 days). The 4% PFA inactivated RNases and preserved mRNA integrity, an essential requirement for the FISH experiments. We used ‘QuantiGene ViewRNA ISH Cell Assay kit’ from Affymetrix, and followed the manufacturer’s instructions for all procedures. Cells were permeabilized, digested with proteinase K (to allow target accessibility), and hybridized with a probe set (20 oligonucleotide pairs, with each pair binding to adjacent regions of the target mRNA). By a proprietary ‘branched DAN’ technology, only oligonucleotides binding to adjacent regions of target mRNA could be hybridized with Pre-amplifier in the next step. Hybridization with Pre-amplifier was followed by hybridization with Amplifier and then fluorophore-conjugated label probe. This sequential signal amplification (up to 8000 fold) achieved high-specificity and high-sensitivity of mRNA detection in single cells.

**7. Patch clamp experiments**

Whole cell currents were recorded from canine or SHR ventricular myocytes at 34°C in the ‘ruptured-patch’ mode, or from COS-7 cells expressing KCNQ1 or KCNQ1+KCNE1 at room temperature in the ‘perforated-patch’ mode with amphotericin B. During recording cells were superfused with normal Tyrode’s solution. To record \( I_{\text{Ks}} \) from canine ventricular myocytes, we applied 4AP (3 mM), CdCl\(_2\) (0.3 mM), and dofetilide (0.5 μM) to suppress overlapping transient outward, sodium, calcium, and rapid delayed rectifier currents. To separate \( I_{\text{Ks}} \) from other overlapping currents in SHR ventricular myocytes, we first recorded total currents (\( I_{\text{total}} \)). Then we applied HMR1556, 10 μM, to totally suppress \( I_{\text{Ks}} \) (I\(_{\text{HMR}}\)). The difference current, \( I_{\text{total}}-I_{\text{HMR}}\), represents \( I_{\text{Ks}} \). Pipette solution contained (mmol/L): K-aspartate 120, KCl 20, ATP 10, EGTA 10, HEPES 10, MgCl\(_2\) 1, pH 7.3. The pipette tip potential was zeroed before forming the whole-cell recording configuration, and the liquid junction potential between the pipette solution and Tyrode’s (∼ -10 mV, pipette side negative) was compensated during off-line data analysis.

Voltage clamp was controlled by Clampex of pClamp10, and the communication between patch clamp amplifier (Axopatch 200B) and computer was through DigiData 1440A. Series resistance compensation was set at 95%. High-frequency noise in the current data was reduced by setting the corner frequency of a low-pass filter (Frequency Devices) at 1 kHz. Voltage clamp protocols
are diagrammed in the figures or described in figure legend. To construct activation curves, the relationship between test pulse voltage ($V_t$) and peak amplitudes of tail currents ($I_{tail}$) was fit with a simple Boltzmann function: $I_{tail} = I_{max}/(1+\exp((V_{0.5}-V_t)/k))$, where $I_{max}$, $V_{0.5}$ and $k$ are estimated maximal tail current amplitude, half-maximum activation voltage, and slope factor. Activation curve was constructed by dividing $I_{tail}$ by $I_{max}$, as ‘fraction activated’, and plotted against $V_t$.

Patch clamp data were analyzed using the following programs: Clampfit of pClamp 10, PeakFit, Microsoft Excel, and SigmaPlot11.2.

8. **Antibodies**

Antibodies targeting the following proteins were used: KCNQ1 (goat pAb from Santa Cruz Biotechnology, cat# sc-10646, or rabbit pAb from Alomone, cat# APC-022), KCNE1 (mouse mAb from AbNova, cat# H00003753-MO1, or rabbit pAb from Alomone, cat# APC-163), GFP (goat pAb, Abcam, cat# ab5450), dsRed (rabbit pAb, Clontech, cat # 632496), calnexin (rabbit pAb, Sigma Aldrich, cat# C4731), BiP (mouse mAb, BD Transduction Laboratories, cat# 610978), ERGIC53 (rabbit pAb, Sigma Aldrich, cat# E1031), GM130 (mouse mAb, Abcam, cat# ab1299), βCOP (mouse mAb, Sigma Aldrich, cat# G6160), RyR2 (mouse mAb, Abcam, cat# ab2827), JPH-2 (goat pAb, Santa Cruz Biotechnology, cat# sc-51313), calsequestrin 2 (mouse mAb, Santa Cruz Biotechnology, cat# sc-390999), SERCA2a (mouse mAb, Santa Cruz Biotechnology, cat# sc-53010), Na/Ca exchanger (NCX, mouse mAb, Swant, cat# R3F1), and α1 Na-K pump (mouse mAb, Abcam, cat# ab7671). Alexa-conjugated secondary antibodies were purchased from Invitrogen.
B. Supplemental figures

Fig. S1 Monitor the expression levels of Q1-GFP and E1-dsR relative to their native counterparts in canine ventricular myocytes (CVMs). (A) Immunoblot images of whole cell lysates from CVMs cultured for 36 hr without or with adenoviruses (- or + Adv), probed with antibody targeting GFP (top left) or dsR (top right). The membranes were stripped and reprobed with antibody targeting Q1 (bottom left) or E1 (bottom right). Dotted rectangles mark non-specific bands and were excluded from analysis. Brackets to the right of +Adv lanes indicate Q1-GFP bands (dimer 250 kDa and higher MW oligomer) and native Q1 bands (monomer doublet at 75 kDa, and dimer doublet at 150 kDa), E1-dsR bands (~40 kDa) and native E1 bands (from 20 to 28 kDa, representing differentially glycosylated forms). The band intensities of each protein species were combined for quantification. (B) Data summary. The expression levels of Q1-GFP and E1-dsR normalized to their native counterparts were 0.88±0.29 and 0.40±0.05, respectively. (n): number of independent experiments. (C) Images of Z-projections of sum of immunofluorescence signals from CVMs cultured for 24 hr without or with adenoviruses. IF detected total Q1 or E1 as noted. In the +Adv myocytes the IF signals represented the combined FP-tagged and native counterpart. (D) Data summary. Pixel contents of +Adv cells normalized by the mean value of –Adv cells: Q1-GFP +native Q1 = 1.43±0.07, E1-dsR+native E1 = 1.20±0.04. n(N): number of myocytes (number of animals) analyzed.
Fig. S2 Time-dependent shift in Q1-GFP and E1-dsR distribution patterns expressed in canine ventricular myocytes. (A) Illustration of data analysis. Details are described in Expanded Methods. (B) % of Q1-GFP immunofluorescence signals in the perinuclear zone or in cell periphery. (C) Top: % of cellular area occupied by E1-dsR vesicles. Middle and bottom: % of E1-dsR immunofluorescence signals in the perinuclear zone or in cell periphery. The numbers of hours of adenovirus incubation and numbers of myocytes analyzed (in parentheses) are listed along the abscissa.
Fig. S3 Distribution patterns of Q1-GFP (A) and E1-dsR (B) expressed alone in canine ventricular myocytes incubated with adenovirus for 24 hr.
Fig. S4 Distribution patterns of Q1-GFP and E1-dsR, coexpressed or expressed separately (marked on top), in SHR ventricular myocytes. Q1-GFP and E1-dsR are detected by immunofluorescence (left 3 columns) or by their intrinsic fluorescence (right 2 columns) in myocytes incubated with adenovirus(es) for the durations specified on top.
Fig. S5 In canine ventricular myocytes Q1 traffics from jSR to the lateral surface in response to chronic stress (premature ventricular contractions ‘PVC’ for 3 months) while E1 stays on cell surface. (A) Confocal immunofluorescence images of native KCNQ1 and KCNE1 in myocytes isolated from control or PVC hearts\textsuperscript{11}. (B) SR vs SL separation as described for Fig 5D, showing that KCNQ1 is in SR fraction (based on SR marker, RyR2) of control but shift to SL fraction in PVC. (C) Representative current traces recorded from myocytes isolated from control or PVC hearts, elicited by the diagrammed voltage clamp protocol, designed to separate $I_{Ks}$ from other overlapping currents including $I_{Kr}$\textsuperscript{11}. Arrows denote $I_{Ks}$ tail currents, normalized by cell capacitance (pA/pF).
C. Video legends

Video #1 3D visualization of the distribution patterns of native KCNQ1 and KCNE1 in a guinea pig ventricular myocyte. For this and video 2, myocytes were stained for native KCNQ1 (rabbit pAb/Alexa488 goat anti-rabbit, green), native KCNE1 (mouse mAb/Alexa568 goat anti-mouse, red), sarcolemma/t-tubules, trans-Golgi network and post-Golgi vesicles (Alexa647 wheat germ agglutinin ‘WGA’, magenta), and nuclei (DAPI, blue). Fluorescence signals were acquired sequentially in z-stacks. We used the program ‘Volocity’ to create 3D visualization. Each video shows the following sequence: WGA/DAPI, KCNQ1/DAPI, KCNE1/DAPI, and KCNQ1/KCNE1/DAPI.

Video #2 3D visualization of distribution of native KCNQ1 and KCNE1 in a canine ventricular myocyte.

Video #3 3D visualization of Q1-GFP and E1-dsR expressed in a canine ventricular myocyte incubated with adenoviruses for 18 hr. For this and videos #4 and #5, Q1-GFP and E1-dsR are detected by immunofluorescence (GFP goat pAb/Alexa488 donkey anti-goat, green; dsR rabbit pAb/Alexa568 donkey anti-rabbit, red), and nuclei are stained blue (DAPI). This video shows the following sequence: Q1-GFP/DAPI, E1-dsR/DAPI, and Q1-GFP/E1-dsR/DAPI.

Video #4 3D visualization of Q1-GFP and E1-dsR expressed in a canine ventricular myocyte incubated with adenoviruses for 24 hr. Alexa647 WGA was also used to stain sarcolemma/t-tubules, trans-Golgi network and post-Golgi vesicles. This video shows the following sequence: WGA/DAPI, Q1-GFP/DAPI, E1-dsR/DAPI, and Q1-GFP/E1-dsR/DAPI.

Video #5 3D visualization of Q1-GFP and E1-dsR expressed in a canine ventricular myocyte incubated with adenoviruses for 36 hr. This video shows the following sequence: Q1-GFP/DAPI, E1-dsR/DAPI, and Q1-GFP/E1-dsR/DAPI.

Video #6 3D visualization of distribution patterns of Q1-GFP (intrinsic fluorescence) and GFP mRNA (detected by red fluorophore-conjugated probes targeting GFP mRNA, Affymetrix) in a young SHR ventricular myocyte incubated with Adv-Q1-GFP for 20 hr. Nuclei was stained blue by DAPI.

Video #7 3D visualization of distribution patterns of E1-dsR (intrinsic fluorescence) and dsRed mRNA (detected by green fluorophore-conjugated probes targeting dsR mRNA, Affymetrix) in a young SHR ventricular myocyte incubated with Adv-E1-dsR for 20 hr. Nuclei was stained blue by DAPI.

Video #8 Time lapse images from a ‘fluorescence recovery after photobleaching’ (FRAP) experiment of Q1-GFP expressed in a canine ventricular myocyte. Three ROIs were bleached: myocyte center (with striations), lateral periphery, and cell ends.

SUPPLEMENT REFERENCES


