Distinct gene-specific mechanisms of arrhythmia revealed by cardiac gene transfer of two long QT disease genes, *HERG* and *KCNE1*

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The long QT syndrome (LQTS) is a heritable disorder that predisposes to sudden cardiac death. LQTS is caused by mutations in ion channel genes including HERG and KCNE1, but the precise mechanisms remain unclear. To clarify this situation we injected adenoviral vectors expressing wild-type or LQT mutants of HERG and KCNE1 into guinea pig myocardium. End points at 48–72 h included electrophysiology in isolated myocytes and electrocardiography *in vivo*. HERG increased the rapid component, I_{Kr}, of the delayed rectifier current, thereby accelerating repolarization, increasing refractoriness, and diminishing beat-to-beat action potential variability. Conversely, HERG-G628S suppressed I_{Kr} without significantly delaying repolarization. Nevertheless, HERG-G628S abbreviated refractoriness and increased beat-to-beat variability, leading to early afterdepolarizations (EADs). KCNE1 increased the slow component of the delayed rectifier, I_{Ks}, without clear phenotypic sequelae. In contrast, KCNE1-D76N suppressed I_{Ks} and markedly slowed repolarization, leading to frequent EADs and electrocardiographic QT prolongation. Thus, the two genes predispose to sudden death by distinct mechanisms: the KCNE1 mutant flagrantly undermines cardiac repolarization, and HERG-G628S subtly facilitates the genesis and propagation of premature beats. Our ability to produce electrocardiographic long QT *in vivo* with a clinical KCNE1 mutation demonstrates the utility of somatic gene transfer in creating genotype-specific disease models.

adenovirus | long QT syndrome | *in vivo*

The long QT syndrome (LQTS) is a heritable disorder characterized by prolonged ventricular repolarization and a high risk of sudden cardiac death (1, 2). LQTS has been linked to lesions in various ion channel genes (loss-of-function mutations in *KvLQT1*, *KCNE1*, *HERG*, or *KCNE2* and gain-of-function mutations in *SCN5A*) (3–8). For most given K channel mutants, the effects are attributable either to simple hemiallelic insufficiency or to a dominant-negative effect whereby mutant channels are not only nonfunctional themselves but also multimerize with and cripple the products of the wild-type allele (9–12). Although it would be logical to expect a more severe phenotype in patients with dominant-negative mutations, in which current is greatly suppressed (13), genotype–phenotype correlations are not sufficiently tight to support such a broad generalization; in fact, the mechanisms underlying fatal arrhythmias in any given phenotype remain unclear (2, 14). Understanding these mechanisms not only would contribute to improved therapy for patients with this relatively rare disease, but would also benefit the much larger population with heart failure, in which patients suffer from sudden death due to an acquired form of LQTS (15).

The use of viral gene transfer to manipulate the genetics of a target cell type has become an important technique in cellular and molecular research. We have previously shown the utility of somatic gene transfer in creating gene-specific alterations in ionic currents of native cells *in vitro* (16–19). More recently we have refined the ability to manipulate gene expression in adult heart *in vivo* (20) and demonstrated the utility of this technique by creating gene-specific changes in electrophysiology and in surface electrocardiograms (ECGs) of animals (21). In the present work, we used adenoviral gene transfer in guinea pigs *in vivo* to express wild-type and long QT disease mutants of two potassium channel genes: *HERG,* which encodes the α subunit of I_{Kr}, and *KCNE1,* which encodes minK, an ancillary subunit of I_{Ks}. The HERG mutation (G628S) is located in the pore region, and the KCNE1 mutation (D76N) falls within the C terminus of the minK protein. Both mutations were discovered by genomic analysis of long QT patients (7, 9, 11). Our goal was to dissect the relative contributions of I_{Kr} and I_{Ks} to cardiac repolarization and to understand, in mechanistic detail, how the mutants cause fatal arrhythmias. Second, we intended to identify candidate genes that might be particularly effective in suppressing cardiac arrhythmias due to abnormal repolarization. Our results demonstrate different mechanisms of arrhythmogenesis for the two mutant genes probed. Furthermore, wild-type HERG and KCNE1 differ dramatically in their effects on the electrical activity of cardiomyocytes.

Materials and Methods

The present investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1985) and was performed in accordance with the guidelines of the Animal Care and Use Committee of the Johns Hopkins University.

Plasmid Construction and Adenovirus Preparation. The adenovirus shuttle vectors pAdEGI (17), pAdCGI (20), and pAdC-DBEcR (21) have been described. The full-length coding sequence of *HERG* (kindly supplied by M. Keating, University of Utah, Salt Lake City) was cloned into the multiple cloning site of pAdEGI to generate pAdEGI-HERG. The disease-making point mutation G628S (9) was introduced into HERG by site-directed mutagenesis, creating the vector pAdEGI-HERG-G628S. The full-length coding sequence of human KCNE1 was PCR-amplified from genomic DNA, control-sequenced, and cloned into the multiple cloning site of pAdCGI, to give pAdCGI-KCNE1. The point mutation D76N (7) was introduced into KCNE1 by site-directed mutagenesis, creating the vector

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Abbreviations: LQTS, long QT syndrome; EAD, early afterdepolarization; ECG, electrocardiogram; APD, action potential duration; QTc interval, rate-corrected QT interval.

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pAdCGI-KCNE1-D76N. Adenovirus vectors were generated as described (18, 22).

Animals. Adult guinea pigs (200–250 g) underwent direct intramyocardial adenovirus injection as reported (20, 21, 23). Animals that had received one of the inducible HERG constructs were injected intraperitoneally with 45 mg of the nonsteroidal ecdysone receptor agonist GS-E (kindly provided by Rohm & Haas) as described (21).

Myocyte Isolation and Electrophysiology. Forty-eight to 72 h after intramyocardial injection, guinea-pig left ventricular myocytes were isolated as described (21, 24–25). Experiments were carried out with the use of standard microelectrode whole-cell patch-clamp or perforated patch techniques at 37°C (21, 26).

Cells were superfused with a physiological saline solution containing (in mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 10 glucose, 1 $MgCl_2$, 10 Hepes; pH was adjusted to 7.4 with NaOH. For I_{Ks} and I_{Kr} recordings, $CaCl_2$ was reduced to 100 μM, and $BaCl_2$ (500 μM) and $CdCl_2$ (200 μM) were added to block I_{K1} and I_{CaL}, respectively. For I_{Ks} recordings, the external solution additionally contained 10^{-6} M dofetilide (Pfizer) to block I_{Kr} (27). To determine drug-sensitive I_{Kr}, currents before and after the addition of dofetilide (10^{-6} M) were subtracted. The micropipette electrode solution was composed of (in mM) 125 K-glutamate, 15 KCl, 5 NaCl, 1 $MgCl_2$, 10 Hepes, and 4 Mg-ATP; pH was adjusted to 7.3 with KOH. I_{Ks} and action potentials of KCNE1-, KCNE1-D76N-, and HERG-G628S-infected animals were recorded with the use of the perforated-patch method with amphotericin B (28) at a concentration of 100–150 μg/ml pipette solution. Because control action potentials obtained with the ruptured-patch (APD_{90}, 160.1 ± 8.1 ms, n = 7) and perforated-patch (APD_{90}, 163.7 ± 12.6 ms, n = 5) techniques were not different, they were combined for further analysis. Data were corrected for the measured liquid junction potential of −18 mV.

Repetition intervals of 15 s and 2 s were used, respectively, for I_{Ks} and I_{Kr} recordings. Action potentials were initiated by brief depolarizing current pulses (2–3 ms, 500–800 pA, 105–110% threshold) at 2 Hz if not otherwise indicated. Action potential duration (APD) was measured as the time from the overshoot to the indicated percentage of repolarization. Beat-to-beat APD variability was determined from 20 consecutive action potentials at 2 Hz. The relative refractory period was measured from the APD_{90} of the first action potential to the time of the premature stimulus that elicited a second action potential as described (19).

Electrocardiograms. Surface ECGs were recorded immediately after operation and 48 h after intramyocardial injection as reported (21, 29). The rate-corrected QT interval (QTc) was calculated with Bazett's formula (30).

Statistical Analysis. Pooled data are presented as mean ± SEM. Comparisons between groups were made with the use of one-way ANOVA. Postoperative and 48-h control QTc intervals were compared with the use of the paired t test. P values less than 0.05 were deemed significant.

Results

To probe the role of the LQTS disease genes *HERG* and *KCNE1* in cardiac repolarization, we expressed wild-type or LQT mutants of *HERG* and *KCNE1* in guinea pigs by *in vivo* gene transfer. Guinea-pig myocardium was injected with wild-type or mutant (D76N) (7) KCNE1 genes in a vector that also expresses the enhanced green fluorescent protein under the control of a single cytomegalovirus promoter, or vectors that express wild-type or mutant (G628S) (4) HERG genes and enhanced green fluorescent protein under the control of an ecdysone inducible

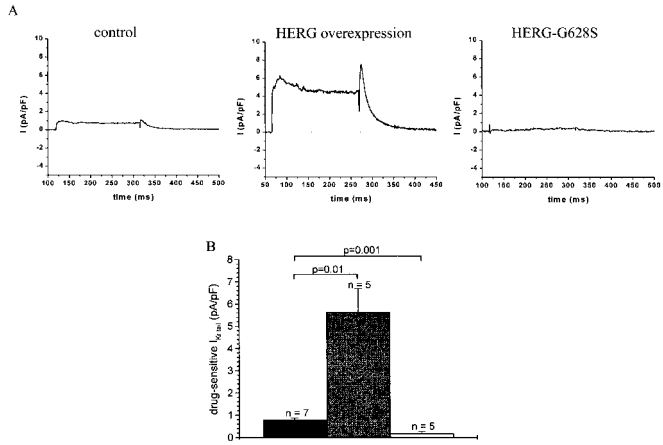

Fig. 1. Drug-sensitive I_{Kr} current in control guinea-pig ventriculocytes compared with myocytes that were infected *in vivo* with AdEGI-HERG or AdEGI-HERG-G628S. Original current traces of the drug-sensitive I_{Kr} (*A*) and pooled data of drug-sensitive I_{Kr} tail current density (*B*) (recorded with 200-ms depolarization pulses to 40 mV followed by repolarization to −50 mV) demonstrate that enhanced expression of HERG (gray bars) significantly increased I_{Kr} compared with noninfected myocytes (black bars), whereas *in vivo* expression of HERG-G628S (white bars) suppressed I_{Kr} tail current density.

promoter (31). Ecdysone responsiveness was conferred by coinjection with the receptor virus AdC-DBEcR and stimulation with the nonsteroidal ecdysone analog GS-E. Forty-eight to 72 h after injection, ECGs were recorded or myocytes were isolated, and infected cells were identified by their bright green fluorescence (clearly distinguishable from background autofluorescence) as described (21).

HERG Overexpression Stabilizes Cardiac Repolarization. Expression of exogenous HERG in guinea pig myocytes resulted in a ≈7-fold increase in drug-sensitive I_{Kr} density. Representative current records (Fig. 1*A*) and pooled data (Fig. 1*B*) show that drug-sensitive I_{Kr} tail current density (measured at −50 mV after a 200-ms depolarization pulse to 40 mV; 5.6 ± 1.1 pA/pF, n = 5; P = 0.01) was significantly increased in HERG-infected myocytes compared with noninfected cells (0.8 ± 0.1 pA/pF, n = 7). HERG overexpression had no effect on the deactivation kinetics of I_{Kr} tail currents (τ_1 = 17 ± 2 ms, P = 0.20; τ_2= 175 ± 50 ms, P = 0.98; at −50 mV after a 200-ms depolarization pulse to 40 mV) compared with noninfected cells (τ_1 = 23 ± 4 ms, τ_2 = 172 ± 76 ms) and thus were faster than described for the expression of HERG in heterologous systems (4, 8, 19, 32, 33). The N-terminal alternatively spliced variants of ERG1 (ERG1b), which exhibit fast deactivation kinetics (34), may not be expressed at the protein level in myocardium (35). Thus, the fast deactivation of overexpressed HERG that we have observed most likely reflects coassembly with endogenous KCNE2 (8). Inactivation, quantified as the ratio of the instantaneous current and the maintained current at the end of the 200-ms depolarization pulse to 40 mV, and recovery from inactivation, quantified as the ratio of peak tail current at −50 mV and the maintained current at the end of the 200-ms depolarization pulse to 40mV, were not significantly different between overexpressed HERG (1.3 ± 0.1 and 1.5 ± 0.1, respectively; n = 6) and control currents (1.3 ± 0.1 and 1.3 ± 0.1, respectively; n = 7). Action potential recordings in HERG-infected myocytes revealed clear-cut effects of the I_{Kr} increase on cardiac repolarization. The representative records in Fig. 2*A* and pooled data in Fig. 2*D* clearly show that increased I_{Kr} substantially prolonged the relative refractory period of HERG-infected myocytes compared with noninfected cells (37.4 ± 7.7 ms, n = 11 vs. control

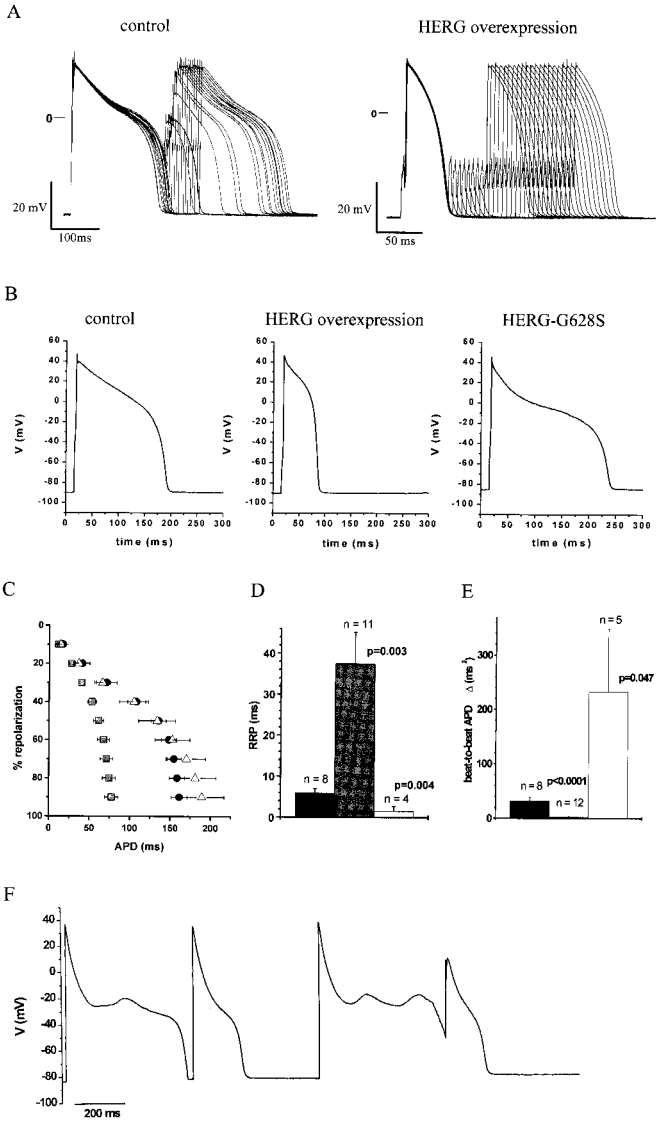

Fig. 2. Effect of *in vivo* expression of HERG wild type and HERG-G628S on cardiac repolarization in freshly isolated guinea-pig myocytes. Original action potential recordings (*A* and *B*) and pooled data (*C–E*) demonstrate that enhanced expression of HERG (gray) substantially prolonged the relative refractory period (*A* and *D*), abbreviated overall APD without changing the action potential waveform (*B* and *C*), and resulted in a very stable action potential with hardly any beat-to-beat APD variability compared with controls (black) (*A* and *E*). I_{Kr} suppression by HERG-G628S (white) resulted only in a nonsignificant APD prolongation (*B* and *C*). Nevertheless, HERG-G628S infection abbreviated the relative refractory period (*D*) and markedly increased beat-to-beat APD variability (*E*), leading to arrhythmogenic EADs (*F*).

5.9 ± 1.1 ms, $n = 8$; $P = 0.003$). The I_{Kr} increase resulted in a very stable action potential with negligible beat-to-beat APD variability [2.0 ± 1.2 ms^2, $n = 12$, vs. control, 32.2 ± 6.4 ms^2, $n = 8$; $P < 0.0001$] (Fig. 2 *A* and *E*). Moreover, HERG overexpression abbreviated overall APD quantified at 90% repolarization (APD_{90} 77.3 ± 8.2 ms, $n = 14$, vs. control, 161.3 ± 9.9 ms, $n = 12$; $P < 0.0001$) without changing the action potential waveform (Fig. 2 *B* and *C*). The combination of these effects (decreased APD variability and increased refractoriness) indicates that enhanced expression of HERG might be particularly beneficial for stabilization of cardiac excitability in disease states with altered repolarization.

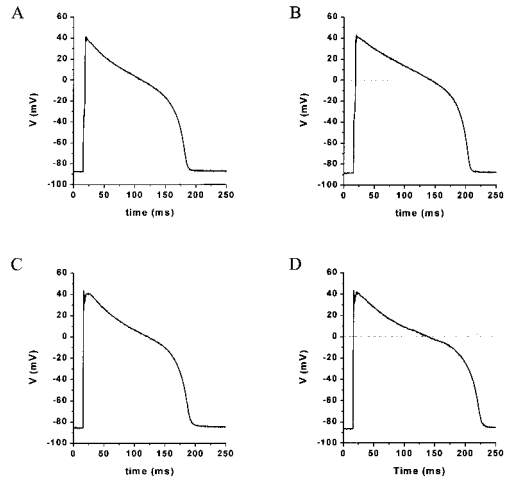

Fig. 3. Original action potential recordings of a HERG-G628S-infected myocyte at 0.5 Hz (*D*) and 2 Hz (*C*) compared with a control cell at 0.5 Hz (*B*) and 2 Hz (*A*) demonstrate that the rate-dependent increase in APD was not significantly different between the mutant and the control. Action potential duration at 0.5 Hz was not significantly longer in HERG-G628S-infected myocytes than in the noninfected cells.

HERG-G628S Alters Action Potential Stability. The disease mutant HERG-G628S, which has been reported to function as a strong dominant-negative gene product (9), significantly and uniformly suppressed drug-sensitive I_{Kr} tail current density (0.14 ± 0.09 pA/pF, $n = 5$; $P = 0.001$) (Fig. 1). Although mean APD was not significantly prolonged by this level of I_{Kr} reduction (APD_{90} 189.2 ± 27.5 ms, $n = 6$; $P = 0.14$) (Fig. 2 *B* and *C*), there was a significant increase in APD variability from cell to cell ($P = 0.01$). HERG-G628S infection also abbreviated the relative refractory period (1.5 ± 1.2 ms, $n = 4$; $P = 0.004$) (Fig. 2*D*) and markedly increased beat-to-beat APD variability (232.6 ± 116.1 ms^2, $n = 5$; $P = 0.047$) compared with noninfected cells (Fig. 2*E*). Fig. 2*F* demonstrates that action potential duration in HERG-G628S-infected myocytes varied extensively and led to early afterdepolarizations (EADs) in every other beat (EADs in one of six cells at 2 Hz, one of three cells at 0.5 Hz). EADs occurred in HERG-G628S-infected myocytes that exhibited longer APDs and a prolonged plateau phase compared with mean values of control myocytes (Fig. 2*F*). EADs were never observed in any noninfected myocytes. A rate-dependent increase in APD at 0.5 Hz vs. 2 Hz was not significantly different between HERG-G628S-infected myocytes (APD_{90} 219.5 ± 23.9 ms and 185.8 ± 21.7 ms at 0.5 Hz and 2 Hz, respectively; increase in APD_{90} 19.0 ± 3.4%, $n = 4$) and noninfected cells (APD_{90} 180.8 ± 6.9 ms and 162.0 ± 7.4 ms at 0.5 Hz and 2 Hz, respectively; increase in APD_{90} 11.5 ± 2.5%, $n = 4$). The action potential duration at 0.5 Hz was not significantly longer in the mutant compared with controls ($P = 0.2$) (Fig. 3). To probe the effect of this LQTS mutant on the surface ECG *in vivo*, guinea-pig myocardium was injected with HERG-G628S at multiple sites [as described (21)]. Consistent with action potential recordings in isolated myocytes, HERG-G628S did not prolong the QT interval of infected animals 48 h after injection compared with immediate postoperative recordings (QTc 305.0 ± 11.4 ms vs. 293.0 ± 9.5 ms, $n = 3$; $P = 0.38$) but did alter the T-wave morphology, possibly because of inhomogeneous expression throughout the ventricles (Fig. 4 *A* and *B*).

KCNE1 Overexpression Increases I_{Ks} without Affecting Repolarization. To study the effect of *in vivo* adenovirus-mediated overexpression of KCNE1 on I_{Ks}, tail currents were recorded at −50 mV after 5-s depolarizing steps from 60 mV to −20 mV. Represen-

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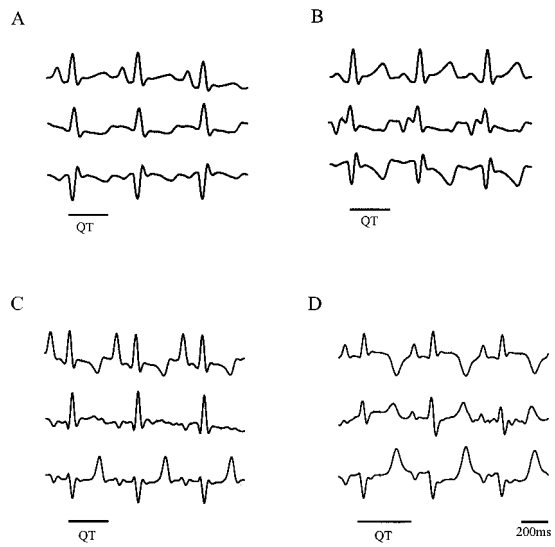

Fig. 4. The LQTS mutant KCNE1-D76N prolongs the QT interval in guinea pigs *in vivo*, whereas HERG-G628S does not affect QT interval duration. ECG recordings were performed after widespread myocardial infection with HERG-G628S or KCNE1-D76N. ECGs revealed a prolongation by ≈23% of QTc 48 h after KCNE1-D76N infection (*D*) compared with immediate postoperative recordings (*C*) (QTc 286.7 ± 10.4 ms vs. 353.3 ± 24.5 ms, $n = 3$; $P = 0.04$), consistent with the marked prolongation of action potentials in KCNE1-D76N-infected ventriculocytes. Conversely, no change in QTc was observed 48 h after infection with HERG-G628S (*B*) compared with immediate postoperative ECGs (*A*) (305.0 ± 11.4 ms vs. 293.0 ± 9.5 ms, $n = 3$; $P = 0.38$), in agreement with the overall unchanged APD of HERG-G628S-infected cells.

tative current records from noninfected and KCNE1-infected myocytes (Fig. 5*A*) and pooled data (Fig. 5*B*) demonstrate that enhanced expression of KCNE1 increased I_{Ks} tail current density at −50 mV after depolarization to 60 mV by ≈60% (11.2 ± 1.1 pA/pF, $n = 11$, vs. 7.0 ± 0.6 pA/pF, $n = 18$; $P < 0.0001$), indicating a regulatory effect of exogenous KCNE1 on I_{Ks}. The underlying mechanism is unclear. To determine whether the I_{Ks} increase resulted from a conversion of unpaired KvLQT1 background current to a time-dependent current (37), we quantified current amplitude 50 ms after the onset of a depolarizing step to 20 mV. Current density at this early time point of depolarization was not different between KCNE1-infected and noninfected myocytes (3.8 ± 0.3 pA/pF, $n = 6$, vs. 3.2 ± 0.5 pA/pF, $n = 14$; $P = 0.62$). This similarity in current density supports the idea that KCNE1 either recruits a dormant KvLQT1 pool or, by complexing with KvLQT isoform 2, reduces the putative dominant-negative effect of isoform 2 (38). Given that different activation kinetics have been proposed for the KCNE1-KvLQT1 complex (39, 40), we measured the time for 10%, 30%, and 50% activation of maximal I_{Ks} current size (at 5,000 ms, 60 mV). *In vivo* overexpression of KCNE1 had no significant effect on I_{Ks} activation kinetics (10%, 30%, and 50% current size at 128 ± 16 ms, 296 ± 23 ms, and 605 ± 41 ms, respectively) compared with noninfected cells (10%, 30%, and 50% current size at 123 ± 22 ms, 346 ± 15 ms, and 711 ± 37 ms, respectively; P = not significant). Enhanced expression of KCNE1 did not affect drug-sensitive I_{Kr} tail current density (0.85 ± 0.23 pA/pF, $n = 5$; $P = 0.83$) (Fig. 5*C*). The I_{Ks} increase by KCNE1 overexpression had no significant effect on overall APD (APD_{90} 154.3 ± 10.4 ms, $n = 9$; $P = 0.49$) (Fig. 6 *A* and *B*), relative refractory period (7.0 ± 1.2 ms, $n = 5$; $P = 0.43$), or beat-to beat APD variability [38.6 ± 12.3 ms^2, $n = 9$; $P = 0.67$] (Fig. 6*C*) compared with noninfected myocytes.

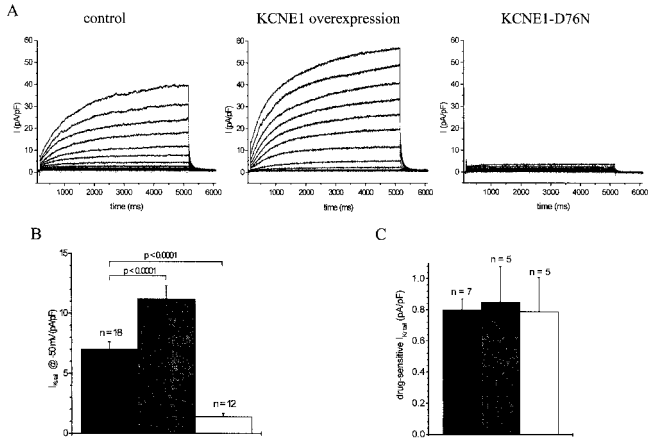

Fig. 5. I_{Ks} and I_{Kr} current density in control guinea-pig ventriculocytes compared with myocytes that were infected *in vivo* with AdCGI-KCNE1 or AdCGI-KCNE1-D76N. (*A*) Original current traces recorded with 5-s depolarizing steps from 60 mV to −20 mV demonstrate that enhanced expression of KCNE1 increased I_{Ks} current size, whereas KCNE1-D76N substantially suppressed I_{Ks}. (*B*) Mean I_{Ks} tail current density measured at −50 mV after depolarization to 60 mV was increased by KCNE1 overexpression (gray bar) by ≈60% and reduced by KCNE1-D76N (white bar) by ≈80% compared with noninfected cells (black bar). (*C*) I_{Kr} drug-sensitive tail current density was measured as described in Fig. 1. We did not observe any effect of KCNE1 overexpression (gray bars) or KCNE1-D76N (white bars) compared with noninfected myocytes (black bars) on drug-sensitive I_{Kr} tail current density in guinea-pig myocardium.

KCNE1-D76N Flagrantly Delays Cardiac Repolarization. Conversely, *in vivo* expression of the disease-causing mutant KCNE1-D76N suppressed I_{Ks} tail current density by ≈80% (1.4 ± 0.3 pA/pF, $n = 12$; $P < 0.0001$) (Fig. 5 *A* and *B*). We did not observe any effect of KCNE1-D76N on drug-sensitive I_{Kr} tail current density (0.79 ± 0.22 pA/pF, $n = 5$; $P = 0.88$) in guinea pig myocardium (Fig. 5*C*). I_{Ks} suppression by KCNE1-D76N prolonged overall APD by almost 2-fold (APD_{90} 277.1 ± 58.7 ms, $n = 7$; $P = 0.02$) (Fig. 6 *A* and *B*) and resulted in a dramatic increase in beat-to-beat APD variability [602.8 ± 248.7 ms^2, $n = 4$; $P = 0.007$] (Fig. 6*C*). This increase in APD variability was associated with frequent EADs, which were observed in all but one myocyte studied at a slow pacing rate of 0.5 Hz, with some cells not repolarizing at all (four of five cells), and in two of eight cells at 2 Hz (Fig. 6*D*). Consistent with this profound APD prolongation in isolated ventriculocytes, KCNE1-D76N prolonged the QT interval in surface ECGs recorded 48 h after widespread myocardial infection, compared with immediate postoperative recordings, by ≈23% (QTc 286.7 ± 10.4 ms vs. 353.3 ± 24.5 ms, $n = 3$; $P = 0.04$) (Fig. 4 *C* and *D*).

Discussion

We have used somatic gene transfer to express wild-type and LQTS disease mutants of the potassium channel genes *HERG* and *KCNE1* in adult guinea-pig heart *in vivo*. Our results show that the two mutant genes exhibit different mechanisms of arrhythmogenesis: the HERG mutation perturbs the regularity and stability of the electrical heartbeat without altering its overall duration, and the KCNE1 mutant sharply delays repolarization as its primary effect. The two wild-type genes also differ dramatically in how well they stabilize electrical activity.

Individuals with LQTS mutations have a high propensity for malignant tachyarrhythmias and sudden cardiac death. However, the QT interval in surface ECGs is not a very reliable predictor for the risk of cardiac events. In fact, unexpected deaths have occurred in carriers of LQTS mutants despite a normal QTc, and QT prolongation is often absent in known carriers of disease genes (1, 2). The more subtle effects of HERG-G628S on cardiac repolarization, despite its lack of effect

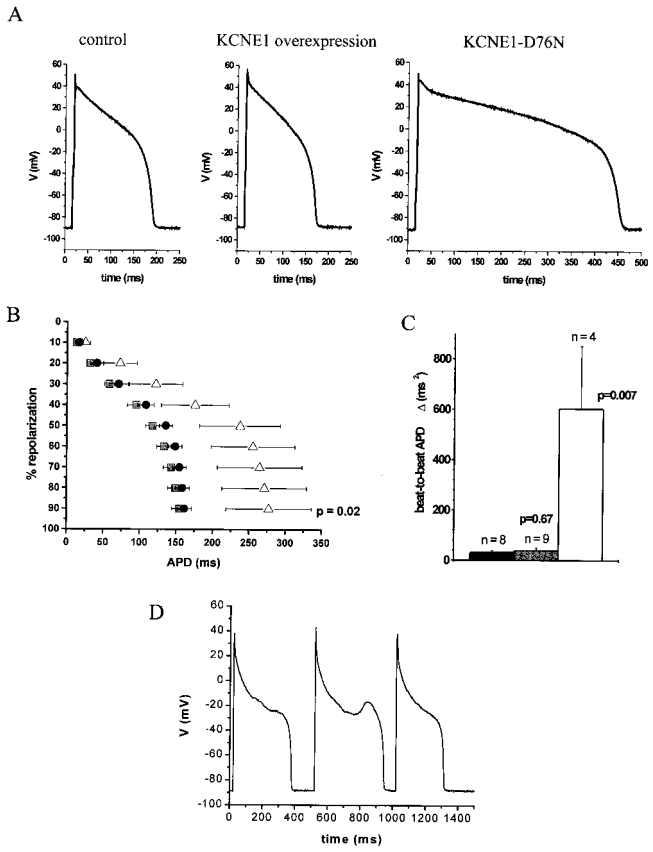

Fig. 6. Effect of *in vivo* expression of KCNE1 wild-type and KCNE1-D76N on cardiac repolarization in freshly isolated guinea pig myocytes. I_{Ks} increase by KCNE1 overexpression (gray) exhibited no significant effect on overall APD (APD_{90} 154.3 ± 10.4 ms, $n = 9$; $P = 0.49$) (*A* and *B*) or beat-to beat APD variability (*C*) compared with noninfected myocytes (black). However, I_{Ks} suppression by KCNE1-D76N (white) prolonged overall APD almost 2-fold (APD_{90} 277.1 ± 58.7 ms, $n = 7$; $P = 0.02$) (*A* and *B*) and resulted in an extensive increase in beat-to-beat APD variability (*C*), which led to frequent arrhythmogenic EADs (*D*).

on the QT interval observed in the present study, provide a cellular rationale for these clinical observations.

Expression of HERG-G628S led to uniform dominant-negative suppression of I_{Kr} in infected cells. Although I_{Kr} suppression did not prolong mean APD, HERG-G628S infection resulted in an increased APD variability. This difference in effect on APD is consistent with the observation that M-cells exhibit more APD prolongation in response to I_{Kr} blockers than epicardial and endocardial myocytes (41). Nonuniform effects on APD by HERG-G628S might lead to alterations of the T-wave morphology and increase dispersion of repolarization, which in fact has been linked to the risk for cardiac events in LQTS patients (42). In addition to these potential different regional effects, HERG-G628S obviously destabilized repolarization in individual cells, as reflected by the markedly increased beat-to-beat APD variability and decreased relative refractory period as well as the increased frequency of EADs. EADs are caused by reactivation of L-type calcium currents and are believed to be a cellular mechanism for the initiation of torsades de pointes and related polymorphic ventricular tachycardias (43–47). HERG-G628S-infected myocytes exhibiting arrhythmogenic EADs had longer APDs compared with mean control. These APDs exhibited a prolongation of the plateau phase in the potential range where I_{CaL} can reactivate. This indicated a reduced net repolarizing current, which makes the plateau less stable (Fig. 2). EADs in HERG-G628S-infected cells were recorded at both fast and slow stimulation rates, and rate-dependent APD prolongation was not significantly different from that in control myocytes. This observation might suffice to explain the clinical observation that rate-dependent QT changes in LQT2 patients are similar to those in healthy controls, and that patients with LQT2 experience cardiac events at rest as well as during exercise (42, 48).

The LQTS mutant KCNE1-D76N markedly reduced I_{Ks}, indicating that KCNE1-D76N exhibits a dominant-negative mechanism in myocardial tissue *in vivo*. These findings are not unexpected, given the previously reported suppression of I_{Ks} by KCNE1-D76N (7, 49). Furthermore, KCNE1 has been proposed to modulate HERG in the heterologous expression system: KCNE1 increased HERG current amplitude without altering cell-surface expression or current kinetics (50), and KCNE1-D76N reduced HERG current density (49). KCNE1 knockout mice have yielded conflicting results; Drici *et al.* (51) recording unchanged I_{Kr} compared with control animals, and Kuperschmidt *et al.* (52) observed a reduction of I_{Kr} in their mice. However, the latter animals also exhibited a change in I_{Kr} deactivation kinetics, raising the possibility that KCNE1 knockout might affect the expression of genes other than HERG itself during development. In the present study, we did not observe any effect on I_{Kr} density by *in vivo* expression of either KCNE1 wild-type or KCNE1-D76N. These inconsistencies between heterologous systems and native heart tissue might reflect the additional presence of KCNE2 in native myocardium. *In vitro*, the presence of both KCNE1 and KCNE2 favored the formation of stable HERG/KCNE2 complexes in preference to those with KCNE1 (8). Thus, the effects of KCNE1 overexpression and dominant-negative suppression on cardiac repolarization in guinea pig myocardium are attributable only to changes in I_{Ks} and not I_{Kr}.

I_{Ks} suppression significantly prolonged the QT interval in surface ECGs *in vivo*. Most cardiac events in patients with LQT1 are related to fast heart rate during exercise or emotional stress (42). In isolated cells we observed a dramatic delay of repolarization at fast stimulation frequency that resulted in extensive beat-to-beat APD variability and frequent EADs. These observations provide a cellular mechanism for arrhythmogenesis in LQT1 patients. In the clinical setting in response to β-adrenergic stimulation during stress, an even more pronounced imbalance between depolarizing I_{CaL} and suppressed I_{Ks} would further favor depolarizations during the plateau phase. At slow stimulation frequency, where no accumulation of I_{Ks} can be expected, KCNE1-D76N infection entirely undermined repolarization in guinea-pig cells: after a few beats myocytes did not repolarize at all. This lack of myocyte repolarization indicated that accumulation of the small remaining I_{Ks} was required to allow repolarization and that I_{Ks} plays the dominant role in the repolarization process in guinea-pig ventricular myocardium. This conclusion is consistent with predictions from numerical simulations (47, 53). However, I_{Ks} suppression will not necessarily entirely undermine repolarization at a slow heart rate in humans, as the relative contribution of different ionic currents to repolarization is variable between species.

QTc prolongation in KCNE1-D76N-infected guinea pigs demonstrates the capacity of somatic gene transfer to create gene-specific models of the LQTS in animals other than transgenic mice, in which the delayed rectifier current plays only a limited role in repolarization (54). The somatic gene transfer approach will enable tests of the arrhythmogenic potential of distinct LQTS mutants in various larger animals with different relative contributions of I_{Ks} and I_{Kr} in cardiac repolarization (55) and electrophysiological properties that might be even closer to those in humans. The present study was designed to prove the principle that mutation-specific LQTS animal models can be created by gene transfer; a logical next step would be to examine long-term survival and the risk for arrhythmias in these animals. Although survival rate was not

an end point in this study, we did observe an increased mortality (by ≈25% over 72 h) in KCNE1-D76N-infected animals.

Therapeutic options for patients with malignant arrhythmias are still limited. Implantable cardioverter defibrillators may be effective in terminating tachyarrhythmias but do not prevent their onset; antiarrhythmic agents are often ineffective and may even be proarrhythmic (56, 57). Our approach can obviously be extrapolated quite directly, at a conceptual level, to gene therapy for disorders of cardiac repolarization. We found that a KCNE1-induced increase in I_{Ks} produced no significant effect on repolarization, which might have been due in part to slowed I_{Ks} activation kinetics. Conversely, I_{Kr} increase by HERG overexpression significantly abbreviated APD and prolonged the relative refractory period, consistent with previous findings in cultured rabbit cells (19). Enhanced expression of HERG also markedly decreased beat-to-beat APD variability. The conjunction of these effects makes HERG a promising candidate gene in the stabilization of cardiac excitability in arrhythmogenic disorders such as heritable LQTS and heart failure. In acquired and heritable LQTS with mutations other than dominant-negative HERG mutations, enhanced expression of HERG might compensate for the impaired ion channel functions, thereby preventing the onset of arrhythmias. Given recent *in vitro* results (36), even in patients with dominant-negative HERG mutations, expression of exogenous HERG might tilt the balance in favor of the wild-type channel and thus might result in physiological I_{Kr} current size. Alternatively, the redundancy of K channels may be exploited: a gene other than HERG (e.g., KvLQT1) might be fruitfully overexpressed in HERG dominant-negative patients. Selective pharmacological gene induction or homogeneous, widespread somatic gene transfer might achieve such a gene-specific therapeutic strategy. Further studies in heart failure and LQTS animal models are warranted to assess the ability of HERG and other K channel genes to suppress increased cardiac excitability without adversely affecting contractility or increasing the risk for reentrant arrhythmias.

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