Original Article

Slow Delayed Rectifier Potassium Current Blockade Contributes Importantly to Drug-Induced Long QT Syndrome

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Background—Drug-induced long QT syndrome is generally ascribed to inhibition of the cardiac rapid delayed rectifier potassium current ($I_{Kr}$). Effects on the slow delayed rectifier potassium current ($I_{Ks}$) are less recognized. Triggered by a patient who carried the K422T mutation in KCNQ1 (encoding the α-subunit of the $I_{Ks}$ channel), who presented with excessive QT prolongation and high serum levels of norfluoxetine, we investigated the effects of fluoxetine and its metabolite norfluoxetine on $I_{Ks}$.

Methods and Results—ECG data from mutation carriers and noncarriers revealed that the K422T mutation per se had mild clinical effects. Patch clamp studies, performed on HEK293 cells, showed that heterozygously expressed K422T KCNQ1/KCNE1 channels had a positive shift in voltage dependence of activation and an increase in deactivation rate. Fluoxetine and its metabolite norfluoxetine both inhibited $I_{Ks}$, with norfluoxetine being the most potent. Moreover, norfluoxetine increased activation and deactivation rates. Computer simulations of the effects of norfluoxetine on $I_{Kr}$ and $I_{Ks}$ demonstrated significant action potential prolongation, to which $I_{Ks}$ block contributed importantly. Although the effects of the mutation per se were small, additional $I_{Ks}$ block by norfluoxetine resulted in more prominent QTc prolongation in mutation carriers than in noncarriers, demonstrating synergistic effects of innate and drug-induced $I_{Ks}$ blockade on QTc prolongation.

Conclusions—$I_{Ks}$ blockade contributes importantly to drug-induced long QT syndrome, especially when repolarization reserve is reduced. Drug safety tests might have to include screening for $I_{Ks}$ blockade. (Circ Arrhythm Electrophysiol. 2013;6:1002-1009.)

Key Words: drug-induced long QT syndrome | fluoxetine | long QT syndrome | potassium channels | torsade de pointes

The ability of drugs to prolong the QT interval on the ECG is one of the leading causes of drug withdrawal from the market.1 Similar to its hereditary forms, drug-induced long QT syndrome (LQTS) may culminate in polymorphic ventricular tachycardia (torsade de pointes, TdP) and ventricular fibrillation, which cause syncope and sudden death.2-5 Drug-induced LQTS is commonly ascribed to blockade of the fast component of the delayed rectifier current ($I_{Kr}$) as a result of a pharmacodynamic interaction of the drug with cardiac potassium channel proteins.4,5 Accordingly, compounds under drug development are routinely screened for their (undesired) ability to block $I_{Kr}$.6 Yet, anecdotal reports indicate that inhibition of slow delayed rectifier current ($I_{Ks}$) may also contribute to drug-induced LQTS.7,8 This possibility has so far received less recognition.

Clinical Perspective on p 1009

Fluoxetine, a widely prescribed antidepressant, is known as a potential LQTS-inducing drug,9 particularly at high serum levels.10,11 Fluoxetine not only blocks $I_{Kr}$ acutely but also inhibits protein trafficking of the $I_{Ks}$ channel, thus further reducing $I_{Ks}$. Norfluoxetine, the active metabolite of fluoxetine, has similar effects.9 However, the effects of fluoxetine and norfluoxetine on $I_{Ks}$ are unknown. To establish whether $I_{Ks}$ blockade by these drugs contributes to LQTS, we conducted the present study. This study was triggered by a patient who presented with TdP at mildly elevated serum levels of norfluoxetine. Genetic analysis revealed a mutation (K422T) in KCNQ1, which encodes the major protein that carries $I_{Ks}$. This mutation was identified previously, but not studied in detail, in a
clinical study on LQTS patients. Using ECG analysis, cytochrome P450 genotyping, patch clamp analysis, and modeling studies, we provide evidence that $I_{Ks}$ blockade by fluoxetine and norfluoxetine contributes importantly to the TdP-inducing potential of these drugs.

Methods
A detailed description of the methods can be found in the online-only Data Supplement.

Clinical Studies
ECGs were obtained from the index patient (screened for mutations in $KCNQ1$ and $KCNH2$) and 7 relatives (screened for the K422T mutation in $KCNQ1$). The index patient underwent analysis of the serum concentrations of fluoxetine and norfluoxetine as well as genotyping of CYP2D6 for determining the presence of CYP2D6 *3, *4, *5, *6, *7, or *8 alleles, or other CYP2D6 deletions or duplications. Written informed consent was obtained from all participants.

cDNA Constructs, Mutagenesis, and Heterologous Expression
Constructs of wild-type (WT) $KCNQ1$ and $\beta$-subunit $KCNE1$ cloned in pSP64 (kindly provided by Dr M.C. Sanguinetti, University of Utah, UT) were subcloned into the mammalian expression vector pcGII. The $KCNQ1$ K422T mutation was created by using the QuikChange site-directed mutagenesis kit (Stratagene), according to manufacturer’s protocol.

HEK293 cells were transfected with 1 μg of WT-$KCNQ1$ cDNA and 1 μg $KCNQ1$ cDNA (WT-$KCNQ1/KCNE1$), or, to recapitulate a heterozygous state, 0.5 μg of both WT and mutant $KCNQ1$ in addition to 1 μg $KCNE1$ (heterozygously expressed $KCNQ1/KCNE1$, ie, HET-$KCNQ1/KCNE1$).

Electrophysiological Experiments
$KCNQ1/KCNE1$ currents were recorded at 36±0.2°C by the amphotericin-perforated patch clamp technique. Activation and deactivation kinetics of $KCNQ1/KCNE1$ currents were determined by voltage clamp protocols as diagrammed in the associated figure. Current densities were calculated by dividing current amplitudes by cell membrane capacitance. Voltage dependence of activation and the time course of current (de)activation were analyzed as detailed in the online-only Data Supplement. Effects of forskolin, fluoxetine, norfluoxetine, tramadol, and codeine (all Sigma-Aldrich) on $KCNQ1/ KCNE1$ currents were tested by computer simulations using the mid-myocardial version of the ten Tusscher et al human ventricular cell model, as updated by ten Tusscher and Panfilov. The experimentally observed mutant- and drug-induced changes in electrophysiological properties of $KCNQ1/KCNE1$ current were implemented as changes in $I_{Ks}$. Previously reported norfluoxetine-induced $I_{Ks}$ blockade was also implemented, resulting in 72% decrease in $I_{Ks}$ current density at a norfluoxetine concentration of 5 μmol/L. Effects on other transmembrane ion currents were not taken into account.

Statistics
Data are presented as mean±SEM. Statistical comparisons were made as detailed in the online-only Data Supplement. P<0.05 defines statistical significance.

Results
Clinical Studies
A 62-year-old woman presented with repeated syncope. ECGs revealed severe QT prolongation (maximal rate-corrected QT interval [QTc; Bazett formula] 620 ms; Figure 1A), TdP (Figure 1B), and ventricular fibrillation. Her history revealed ventricular tachycardia after minor surgery at age 54 years, which was not explored further. She had hypertension and diabetes. Physical examination and serum electrolytes were unremarkable. Echocardiography revealed mild concentric left ventricular hypertrophy, but was otherwise unremarkable. There were no signs of ischemic heart disease or heart failure. There was a family history of sudden death in 1 relative (father; aged 52 years). Other relatives had no history of LQTS-related symptoms (Figure 2). Her medication included fluoxetine (20 mg OD), which she had used for >2 years, and no other QT-prolonging drugs. Serum levels of fluoxetine and norfluoxetine at admission were 70 and 1230 ng/mL, the latter reaching 1490 ng/mL 1 day after admission (therapeutic ranges are 100–450 and 50–350 ng/mL, respectively). The increased serum levels of norfluoxetine were ascribed to the fact that she had recently started using codeine (15 mg OD) and tramadol (100 mg OD). Both drugs are substrates of cytochrome CYP2D6 and compete with fluoxetine and norfluoxetine as substrates for CYP2D6. On discontinuation of fluoxetine, codeine, and tramadol, QTc duration normalized to 434 ms, and TdP did not recur. Genetic screening for CYP2D6 revealed that she was an extensive metabolizer of CYP2D6 with genotype CYP2D6*1/*1. No additional mutations, deletions, or duplications in these CYP2D6 alleles were found.

Figure 1. Twelve-lead ECG of index patient at admission. Prominent QTc prolongation (maximal QTc interval, 620 ms; A) culminating in an episode of torsade de pointes (25 mm/s; 10 mm/mV; B).
Initial genetic screening for mutations in KCNH2 revealed no mutations. However, she was found to be a heterozygous carrier of a missense mutation in KCNQ1 (K422T), which encodes the pore-forming subunit of the I\textsubscript{Ks} channel. This variant, previously associated with LQTS,\textsuperscript{12} was not found in 400 alleles of control individuals. To assess the clinical consequences of the K422T mutation, 8 family members of the index patient were invited for genetic screening and functional testing (ECGs at rest and exercise ECGs). Of these family members, 7 accepted to undergo genetic screening and ECGs at rest, of whom 5 also accepted to undergo exercise testing. Genetic analysis revealed 3 more carriers of the mutation (Figure 2). At rest, QTc durations were not prolonged in mutation carriers (<450 ms for men and <470 ms for women).\textsuperscript{3} Within generation II, exercise ECGs were analyzed in 2 carriers and the index patient, who demonstrated mild QTc prolongation. QTc intervals remained within normal range in all family members (1 carrier and 2 noncarriers) in generation III. However, given the small family size, the unequal distribution of carriers and noncarriers over the generations, and the difference in genetic relations between carriers and noncarriers, it was impossible to complement these clinical observations with an adequate statistical comparison.

Electrophysiological Studies

Effects of the K422T Mutation on KCNQ1/KCNE1 Current

Figure 3A shows typical currents of WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 channels. The steady-state currents of both channel types did not differ significantly (Figure 3B). Compared with WT-KCNQ1/KCNE1, HET-KCNQ1/KCNE1 channels displayed a +13.3 mV shift (P=0.005) of steady-state activation (Figure 3C). Moreover, in the physiological range from −20 to −70 mV, deactivation was 20% to 25% faster (P<0.05) in HET-KCNQ1/KCNE1 channels (Figure 3E). The reversal potentials (Figure 3D) and activation time constants (Figure 3E) were not significantly different. Because mild QTc prolongation during exercise, found in 3 of 4 mutation carriers, may result from differential responses to β-adrenergic stimulation between HET-KCNQ1/KCNE1 channels and WT-KCNQ1/KCNE1 channels, we studied the effects of forskolin (10 µmol/L), a stimulator of cAMP production, using the protocol shown in Figure 3A. Forskolin significantly increased current density, decreased activation time constant, and shifted V\textsubscript{1/2} (half-maximum activation voltage). However, these effects did not differ significantly between HET-KCNQ1/KCNE1 and WT-KCNQ1/KCNE1 (Figure 4A and 4F).

Effects of Fluoxetine and Norfluoxetine on KCNQ1/KCNE1 Current

Next, we studied the effects of fluoxetine and norfluoxetine on current density and kinetics of WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 channels using the protocols shown in Figure 3A. The index patient experienced QTc prolongation with serum levels of norfluoxetine reaching 1490 ng/mL (≈5 µmol/L). Accordingly, this concentration was used to assess the effects of this drug, whereas the effects of fluoxetine were examined using a supratherapeutic concentration of 10 µmol/L. Figure 4B and 4C shows typical examples of the effects of both drugs on WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 currents, elicited by a voltage clamp step to +110 mV from a holding potential of −80 mV. The average effects are summarized in Figure 4F. Application of 10 µmol/L fluoxetine resulted in 25% blockade, whereas norfluoxetine inhibited I\textsubscript{Ks} by 55% at 5 µmol/L. Both effects did not differ significantly between WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 channels. Norfluoxetine also decreased activation and deactivation time constants at membrane potentials of +110 and −60 mV, respectively (by ≈20% and ≈30%, respectively; Figure 4F). Again, these effects did not differ between WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1.

To study whether the mutation changed the sensitivity to I\textsubscript{Ks} blockade by norfluoxetine, a dose–response curve was obtained by using concentrations between 0.01 and 100 µmol/L (Figure 5). We found that IC\textsubscript{50} values for blocking effects of norfluoxetine on WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 channels were 5.3±0.8 and 4.9±0.8 µmol/L,
with Hill coefficients of 0.78±0.10 and 0.89±0.14, respectively. All effects were reversible on washout (data not shown).

**Effects of Tramadol and Codeine on KCNQ1/KCNE1 Current**

The occurrence of TdP in the index patient coincided with her recent use of tramadol and codeine. Tramadol is not associated with QTc prolongation, whereas codeine and its metabolite morphine are known to have only weak potential of blocking $I_{Kr}$ current, with IC$_{50}$ levels >100 µmol/L above the therapeutic limit of 0.8 µmol/L. To exclude any QTc-prolonging effects of these drugs in the index patient, we evaluated the effects of tramadol and codeine on $I_{Ks}$. Neither tramadol (30 µmol/L; Figure 4D and 4F) nor codeine (30 µmol/L; Figure 4E and 4F) altered the electrophysiological properties of WT-KCNQ1/KCNE1 or HET-KCNQ1/KCNE1 channels.

**Modeling Studies**

Having found that HET-KCNQ1/KCNE1 channels have altered kinetic properties that are predicted to reduce net $I_{Ks}$, and that norfluoxetine also significantly reduced $I_{Ks}$ (in addition to $I_{Kr}$), we next assessed the physiological implications of these changes by conducting modeling studies. Figure 6 shows the simulated action potential at 1 Hz and corresponding $I_{Kr}$ and $I_{Ks}$ currents of a noncarrier (Figure 6A–C; black lines) and a heterozygous K422T mutation carrier (Figure 6D–F; black lines). The mutation prolonged the action potential by 28 ms (Figure 6A and 6D; black lines) as a result of reduction in $I_{Ks}$ (Figure 6C and 6F; black lines). This effect was similar in simulations at higher frequencies (data not shown).

We next studied the effects of inhibition of $I_{Kr}$ and $I_{Ks}$ by 5 µmol/L norfluoxetine. In noncarriers, $I_{Ks}$ inhibition by norfluoxetine prolonged action potential duration by 68 ms (Figure 6A; blue dashed line). Norfluoxetine-induced $I_{Ks}$ inhibition also caused action potential prolongation, albeit less than $I_{Kr}$ inhibition (28 ms; Figure 6A; red dotted line). However, combined $I_{Ks}$ and $I_{Kr}$ inhibition resulted in synergistic effects, prolonging action potential duration by 118 ms (Figure 6A; arrow). In mutation carriers, where $I_{Ks}$ is already...
reduced by the mutation, inhibition of $I_{Kr}$ by norfluoxetine caused more action potential prolongation than in noncarriers (+94 ms), whereas inhibition of $I_{Ks}$ had similar effects (+25 ms; Figure 6D; blue dashed line and red dotted line). Here, inhibition of both $I_{Kr}$ and $I_{Ks}$ caused even larger synergistic effects than in noncarriers, resulting in action potential prolongation by 154 ms (Figure 6D; arrow).

**Discussion**

We found that $I_{Kr}$ blockade contributes importantly to pathological drug-induced QT prolongation. $I_{Kr}$ blockade by norfluoxetine, not previously recognized, although mild in isolation, acted synergistically with inhibition of $I_{Ks}$ to cause marked action potential prolongation. This effect was particularly large in the index patient, who had increased vulnerability to these effects because she carried a $KCNQ1$ mutation that reduced $I_{Ks}$. Our modeling studies indicated that, although $I_{Kr}$ blocking effects of norfluoxetine alone would increase action potential duration by only 68 ms, the additional presence of $I_{Kr}$ block by norfluoxetine and $I_{Ks}$ reduction by the mutation resulted in 154 ms prolongation.

We studied the effects of elevated norfluoxetine concentrations that corresponded to the serum levels found in the index patient. Many situations are conceivable in which the therapeutic levels of fluoxetine and norfluoxetine are exceeded. This report deals with a situation that may occur relatively commonly, that is, drug competition for metabolizing enzymes (CYP2D6). In the index patient, recent concomitant use of tramadol and codeine, drugs that compete with fluoxetine and norfluoxetine as substrates for CYP2D6, presumably increased serum concentrations of norfluoxetine to above-therapeutic levels. Given that neither tramadol nor

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**Figure 4.** Effects of forskolin, fluoxetine, norfluoxetine, tramadol, and codeine on wild-type (WT) and heterozygously expressed (HET) $KCNQ1/KCNE1$ channels. A to E, Typical examples of WT-$KCNQ1/KCNE1$ and HET-$KCNQ1/KCNE1$ currents, activated by depolarizing voltage clamp step to +110 mV, in absence and presence of forskolin (10 µmol/L; A), fluoxetine (10 µmol/L; B), norfluoxetine (5 µmol/L; C), tramadol (30 µmol/L; D), and codeine (30 µmol/L; E). F, Average effects of forskolin (WT, n=7; HET, n=5), fluoxetine (WT, n=7; HET, n=6), norfluoxetine (WT, n=6; HET, n=6), tramadol (WT, n=6; HET, n=4), and codeine (WT, n=5; HET, n=5) on current density, $V_{1/2}$ of activation curve, activation time constant (measured during the depolarizing voltage clamp step to +110 mV), and deactivation time constant (measured at −60 mV after the depolarizing voltage clamp step to +110 mV). *$P<0.05$ (paired $t$ test).
codeine affected repolarizing currents, it is likely that this increase in serum norfluoxetine levels has caused LQTS and TdP/ventricular fibrillation in a patient who had used fluoxetine for >2 years without arrhythmia. This notion was supported by CYP2D6 genotyping, which indicated that she was an extensive metabolizer, not an ultrarapid metabolizer, in whom addition of extra CYP2D6 substrates such as tramadol and codeine would be unlikely to cause relevant increases in norfluoxetine serum levels. Conversely, CYP2D6 genotyping also indicated that this competition may be disease-causing even in extensive metabolizers, such as our index patient. However, although concomitant use of fluoxetine, tramadol, and codeine is probably common, drug-induced LQTS by this drug combination is not commonly reported. We demonstrate that the occurrence of LQTS/TdP required the presence of increased susceptibility to the LQTS-inducing potential of these drugs. In the index patient, such susceptibility was provided by the K422T mutation in KCNQ1.

In the absence of QT-prolonging drugs, the biophysical derangements caused by the K422T mutation were small and did not cause appreciable QTc prolongation. Although slight QTc prolongation was observed during exercise in 3 mutation carriers, no hard conclusions could be drawn on the clinical effects of the mutation per se. QTc interval is known to increase with age, which is a likely explanation for the observed effects here, especially because no QTc prolongation was observed in the investigated carrier in generation III. Also, our electrophysiological studies with forskolin did not show altered responsiveness to \( \beta \)-adrenergic stimulation in mutant channels. The hastened deactivation time course demonstrated in K422T channels could, however, lead to small...
action potential prolongation at fast heart rates, as a consequence of less accumulation of $I_{Ks}$.24

Although the QTc-prolonging effects of K422T mutation per se were limited, our modeling studies indicated that they reduced the repolarization reserve25–27 sufficiently to result in more severe QTc prolongation in the presence of supratherapeutic norfluoxetine levels. These modeling studies also revealed that the $I_{Ks}$-blocking effects of norfluoxetine contribute importantly to the pathological QT prolongation observed here and act synergistically with the well-known $I_{Kr}$-blocking effects of this drug. Taken together, these findings highlight the role of $I_{Ks}$ blockade in the occurrence of drug-induced LQTS. Confirmation in future studies that disease-causing QT prolongation by drugs involves their $I_{Ks}$-blocking properties may have important clinical implications. For instance, in vitro testing in development programs for novel drugs may have to include screening for $I_{Ks}$ blockade liability.28,29 $I_{Ks}$ blockade by such drugs may cause disease-causing QT prolongation in the added presence of common factors that reduce repolarization reserve, for example, gene variants, electrolyte imbalance, or concomitant cardiac disease.

Remarkably, although supratherapeutic levels of fluoxetine and norfluoxetine are clinically associated with QTc prolongation,9–11 previous electrophysiological studies showed action potential shortening in canine-, guinea pig-, and rabbit-isolated myocytes, whereas studies in rat myocytes showed lengthening of the action potential by fluoxetine.15,30–32 These contradictory effects may be explained by species differences in electrophysiological properties. For example, action potential prolongation by inhibition of $I_{Ks}$ is clearly species-dependent, as shown in simulations and experimental studies.31 The observed differences might also be attributed to the long-term effects of norfluoxetine on KCNH2 channel expression. Such effects are obviously not discernible in the aforementioned studies in which the acute effects of fluoxetine or norfluoxetine on action potential duration were tested. Long-term effects of fluoxetine or norfluoxetine on expression of other ion channels have not been assessed thus far and require further investigation.

This study has some limitations. First, the concentration of drugs at the cellular level is hard to predict; although the fact that norfluoxetine is highly protein-bound would obviously lead to lower active levels,34 conversely, tissue accumulation in the heart occurs, resulting in heart/serum concentration ratios of 7±2.35 Second, the computer modeling studies should be interpreted as a qualitative rather than a quantitative illustration. Because we were interested in the contribution of $I_{Ks}$ blockade to action potential prolongation, the effects of norfluoxetine on sodium current, L-type calcium current, and transient outward current, previously shown in isolated canine ventricular myocytes,15 were not taken into account. Furthermore, given the uncertainties in the electrophysiology of human ventricular myocytes and, consequently, in mathematical models of such myocytes, we refrained from performing tissue simulations in order to extrapolate action potential duration data to ECG. Nevertheless, our modeling studies clearly showed an important contribution of $I_{Ks}$ blockade to action potential prolongation and revealed that K422T mutation carriers are more prone to this effect. Third, although we assumed that norfluoxetine serum levels were increased by concomitant use of tramadol and codeine, we could not prove this assumption, because serum levels of norfluoxetine levels before tramadol/codeine use were not available. Finally, although 90% of the LQTS cases are caused by reduction in either $I_{Ks}$ or $I_{Kr}$,3 other gene mutations associated with prolonged QT interval cannot be excluded.

In conclusion, we demonstrate here, on the basis of a patient with $I_{Ks}$ reduction because of a KCNQ1 mutation, that the $I_{Ks}$-blocking effects of the noncardiac drug norfluoxetine contribute importantly to the occurrence of lethal cardiac arrhythmias. These findings highlight the importance of $I_{Ks}$ blockade in drug-induced LQTS.

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Disclosures
None.

References
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CLINICAL PERSPECTIVE

The ability of drugs to prolong the QT interval on the ECG is one of the leading causes of drug withdrawal from the market. In general, this ability is ascribed by blockade of the rapid delayed repolarizing current (I\textsubscript{K}) The effects of slow delayed rectifier current (I\textsubscript{Ks}) blockade are less recognized. This study shows that I\textsubscript{Ks} blockade, in the case of norfluoxetine, contributes importantly to QTc prolongation. Furthermore, certain gene variants, for example, the K422T mutation in KCNQ1, increase susceptibility to this effect. Drug safety tests, which currently only include screening for I\textsubscript{Ks} blockade, may have to screen for effects on I\textsubscript{Ks} as well.
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SUPPLEMENTAL MATERIALS

Supplemental methods

ECG analysis
Twelve-lead ECGs were obtained from the index patient during and after admission, and from 7 relatives. Written informed consent was obtained from all participants. Exercise testing was conducted in 6 individuals, including the index patient. QT durations were corrected for heart rate according to Bazett’s formula.

Genetic analysis
*KCNQ1* and *KCNH2* were screened for mutations as described previously.\(^1\)\(^2\) Genotyping analysis of CYP2D6, with particular interest in determining the presence of CYP2D6 *3, *4, *5, *6, *7 or *8 alleles, or other CYP2D6 deletions or duplications, was performed by means of TaqMan® SNP Genotyping Assays, according to manufacturer’s protocol (Applied Biosystems, Foster City, CA).

cDNA constructs and mutagenesis
Constructs of wild-type *KCNQ1* and β-subunit *KCNE1* cloned in pSP64 were kindly provided by Dr. Michael C. Sanguinetti, University of Utah, Salt Lake City, UT. To transfact and express these constructs, wild-type *KCNQ1* and wild-type *KCNE1* were excised from this vector and subcloned into the mammalian expression vector pCGI. The *KCNQ1*-K422T mutation was created by using the QuikChange site-directed mutagenesis kit (Stratagene, Santa Clara, CA), according to manufacturer’s protocol. Mutant *KCNQ1* was constructed by means of PCR, amplified with primers containing the point mutation K422T. The following oligonucleotides complementary to wild-type gene were used (boldface letters indicate mutation): 5’GTGGTGGTAAAGAAAAAAGCGTTCAAGCTGGAC 3’ (forward) and 5’GTCCAGCTTGAACGTTTTTTTTCTTACCACCAC 3’ (reverse). The mutation was verified and confirmed by DNA sequencing. To exclude point mutations within the plasmid, *KCNQ1*-K422T was excised and cloned back into wild-type pCGI-*KCNQ1* using *HindIII* and *EcoRI* restriction sites. The complete *KCNQ1* gene was sequenced to confirm correctness.

Cell preparation and heterologous expression
HEK293 cells were cultured in Minimal Essential Medium (MEM) supplemented with 10%
fetal bovine serum, penicillin, streptomycin, and non-essential amino acids. To express the \( \text{KCNQ1/KCNE1} \) potassium current of wild-type channels, cells were transiently transfected with lipofectamine using 1 \( \mu \)g of wild-type \( \text{KCNQ1} \) cDNA and 1 \( \mu \)g \( \text{KCNE1} \) cDNA (WT-\( \text{KCNQ1/KCNE1} \)). To recapitulate a heterozygous state, 0.5 \( \mu \)g of both wild-type and mutant \( \text{KCNQ1} \), in addition to 1 \( \mu \)g \( \text{KCNE1} \), were co-transfected (HET-\( \text{KCNQ1/KCNE1} \)). Coexpression of green fluorescent protein (GFP) was realized by an internal ribosomal entry site cassette in the vector. Transfected cells were identified by an epifluorescent microscope. After transfection, the cells were incubated in 5% \( \text{CO}_2 \) at 37°C for 24–48 hours.

**Electrophysiological experiments**

\( \text{KCNQ1/KCNE1} \) currents were recorded at 36±0.2°C by the amphotericin-perforated patch-clamp technique using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Cells were superfused with solution containing (in mM): NaCl 140, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1, glucose 5.5, HEPES 5, pH 7.4 (NaOH). Pipettes of borosilicate glass were filled with solution containing (in mM): K-gluc 125, KCl 20, NaCl 10, amphotericin-B 0.88, HEPES 10, pH 7.2 (KOH) and had resistances of 1.5–2 M\( \Omega \). Series resistance was compensated by ≥80%, and potentials were corrected for the estimated liquid junction potential. Signals were low-pass filtered (cut-off frequency: 2 kHz) and digitized at 2 kHz. Voltage control, data acquisition, and data analysis were accomplished using custom software. Cell membrane capacitance was estimated by dividing the time constant of the decay of the capacitive transient in response to 5 mV hyperpolarizing voltage clamp steps from –60 mV by the series resistance, and amounted to 9.8±0.4 pF (mean±SEM, \( n=124 \)).

The activation and deactivation kinetics of the \( \text{KCNQ1/KCNE1} \) currents were determined by the voltage clamp protocols diagrammed in Figure 3, A and D, respectively. For both protocols, holding potential was –80 mV and the cycle interval 6 seconds. Voltage-dependence of activation was determined from tail currents, which were fitted with a Boltzmann function \( I/I_{\text{max}}=A/(1.0+\exp[(V_{1/2}–V)/k]) \) to determine the half-maximum activation voltage (\( V_{1/2} \)) and slope factor (k). The time course of current activation and deactivation were fitted by the monoexponential equations \( I/I_{\text{max}}=A\times[1–\exp(–t/\tau)] \) and \( I/I_{\text{max}}=A\times\exp(–t/\tau) \), respectively. Current densities were calculated by dividing current amplitudes by the cell membrane capacitance.
Drugs

The effects of forskolin, fluoxetine, norfluoxetine, tramadol, and codeine on KCNQ1/KCNE1 currents were tested 5 minutes after the onset of bath application. Forskolin was prepared as 10 mM stock solution in ethanol. Fluoxetine, norfluoxetine, tramadol, and codeine were prepared as 5 mM stock solutions dissolved in distilled water. All stock solutions were diluted appropriately before use. The effects of the drugs were evaluated in paired measurements at a membrane potential of +110 mV. At this membrane potential the drug effects on density could be assessed without interference by the effects on voltage-dependency of activation. All drugs were obtained from Sigma-Aldrich, St.Louis, MO. Dose-response curves were fitted to the Hill equation $I_{d}/I_{c}=1/[1+(dose/IC_{50})^n]$, where $I_{d}/I_{c}$ is the normalized $I_{Ks}$ current at a membrane potential of +110 mV, dose is the bath concentration of the drug, IC$_{50}$ is the dose required for 50% current block, and n is the Hill coefficient. Serum concentrations of fluoxetine and norfluoxetine were assayed according to a previously described method. 

Computer simulations

Functional differences between WT-KCNQ1/KCNE1 and HET-KCNQ1/KCNE1 were tested by computer simulations using the midmyocardial version of the ten Tusscher et al. human ventricular cell model, as updated by ten Tusscher and Panfilov. This midmyocardial version of the model has a 4-fold lower $I_{Ks}$ density, but is otherwise identical to the generic epicardial version of the model. We selected the midmyocardial version in order to limit the effects of a possible overscaling of $I_{Ks}$ in the ten Tusscher et al. model, as discussed by Grandi et al. and O’Hara et al. 

The experimentally observed mutant- and drug-induced changes in electrophysiological properties of KCNQ1/KCNE1 current were implemented as changes in $I_{Ks}$. The effect of the K422T mutation was implemented as a +13.3 mV shift in the $I_{Ks}$ steady-state activation curve, through a +13.3 mV shift in the $x_{s,c}$-$V_m$ relation of the model, and a 20% increase in deactivation rate $\beta_{xs}$. The inhibitory effect of 5 µM norfluoxetine was implemented as a 55% decrease in current density $G_{Ks}$, a 25% increase in activation rate $\alpha_{xs}$, and a 40% increase in deactivation rate $\beta_{xs}$.

Previously reported norfluoxetine-induced $I_{Ks}$ block was also implemented. When fitted to the Hill equation, norfluoxetine showed an IC$_{50}$ value of 2.5 µM, with a Hill coefficient of 1.3. Accordingly, the effect of 5 µM norfluoxetine on $I_{Ks}$ was implemented as a 72% decrease
in current density $G_K$. Effects of norfluoxetine on other transmembrane ion currents\(^9\) were not taken into account.

Software was compiled as a 32-bit Windows application using Compaq Visual Fortran 6.6C and run on an Intel Xeon processor based workstation. For numerical integration of differential equations we applied a simple and efficient Euler-type integration scheme with a 5-\(\mu\)s time step. All figures show steady-state action potential characteristics, obtained at 2 min after onset of 1-Hz stimulation with a 1-ms, 9.2-nA stimulus.

**Statistics**

Data are presented as mean±SEM. Statistical comparison between WT-\(KCNQ1/KCNE1\) and HET-\(KCNQ1/KCNE1\) was performed by using unpaired t-test, except for the comparison of time constants of activation and deactivation between these groups, which were evaluated by means of Two-Way Repeated Measures ANOVA (RM-ANOVA) followed by pairwise comparison using the Student-Newman-Keuls test. To assess the effects of drugs on WT-\(KCNQ1/KCNE1\) and HET-\(KCNQ1/KCNE1\) channels, paired t-tests were used. P<0.05 defines statistical significance.

**Supplemental references**


