Latent Genetic Backgrounds and Molecular Pathogenesis in Drug-Induced Long-QT Syndrome

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Background—Drugs with $I_{\text{Kr}}$-blocking action cause secondary long-QT syndrome. Several cases have been associated with mutations of genes coding cardiac ion channels, but their frequency among patients affected by drug-induced long-QT syndrome (dLQTS) and the resultant molecular effects remain unknown.

Methods and Results—Genetic testing was carried out for long-QT syndrome–related genes in 20 subjects with dLQTS and 176 subjects with congenital long-QT syndrome (cLQTS); electrophysiological characteristics of dLQTS-associated mutations were analyzed using a heterologous expression system with Chinese hamster ovary cells together with a computer simulation model. The positive mutation rate in dLQTS was similar to cLQTS (dLQTS versus cLQTS, 8 of 20 [40%] versus 91 of 176 [52%] subjects, $P=0.32$). The incidence of mutations was higher in patients with torsades de pointes induced by nonantiarrhythmic drugs than by antiarrhythmic drugs (antiarrhythmic versus others, 3 of 14 [21%] versus 5 of 6 [83%] subjects, $P<0.05$). When reconstituted in Chinese hamster ovary cells, $KCNQ1$ and $KCNH2$ mutant channels showed complex gating defects without dominant negative effects or a relatively mild decreased current density. Drug sensitivity for mutant channels was similar to that of the wild-type channel. With the Luo-Rudy simulation model of action potentials, action potential durations of most mutant channels were between those of wild-type and cLQTS.

Conclusions—dLQTS had a similar positive mutation rate compared with cLQTS, whereas the functional changes of these mutations identified in dLQTS were mild. When $I_{\text{Kr}}$-blocking agents produce excessive QT prolongation (dLQTS), the underlying genetic background of the dLQTS subject should also be taken into consideration, as would be the case with cLQTS; dLQTS can be regarded as a latent form of long-QT syndrome. (Circ Arrhythmia Electrophysiol. 2009;2:511-523.)

Key Words: long-QT syndrome ■ secondary ■ drug ■ electrophysiology ■ ion channel

Congenital long-QT syndrome (cLQTS) is characterized by abnormally prolonged ventricular repolarization and familial inheritance, leading to polymorphic ventricular tachycardia (torsades de pointes [Tdp]), causing sudden cardiac death.$^{1,2}$ In contrast, secondary long-QT syndrome can be induced by a variety of commercially available drugs, including antiarrhythmic drugs, antihistamines, antibiotics, and major tranquilizers.$^3$ In patients with drug-induced long-QT syndrome (dLQTS), after a washout period of the culprit drugs, the QT interval usually returns to within normal range. Genetic factors may underlie the susceptibility to drug-induced serious adverse reactions such as a long QT.

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interval and TdP, Sesti et al. demonstrated that a polymorphism of the KCNE2 gene (T8A) is present in 1.6% of the population and is associated with drug-induced TdP related to quinidine and to sulfamethoxazole/trimethoprim administration. We have also previously reported that a mutant SCN5A channel (L1825P), found in an elderly woman with cisapride-quinidine and to sulfamethoxazole/trimethoprim administration, indicated a low probability of long-QT syndrome, respectively. The present study therefore aimed to survey mutations in long-QT syndrome–related genes responsible for dLQTS in 20 patients who had been referred to our institutes consecutively over the past 11 years and analyze the functional effects induced by these mutations.

Methods

Subjects

Blood samples of 305 subjects with long-QT syndrome, comprising 196 long-QT syndrome probands and 109 family members were referred to Kyoto University Graduate School of Medicine and Shiga University of Medical Sciences from March 1996 to January 2006 for genetic analysis. A diagnosis of dLQTS was made in those subjects who had not previously been diagnosed with long-QT syndrome and who only developed typical ECG features of QT prolongation after administration of culprit drugs. A diagnosis of cLQTS was made in those subjects with clinical phenotypes of long-QT syndrome, but without the involvement of secondary factors (eg, drugs, hypokalemia, or bradycardia). Among the subjects, 20 probands had drug-induced cardiac events (10.2% of dLQTS cohort), however, remains unknown, and the relationship between genotypes and cellular electrophysiology has not been fully examined. The present study therefore aimed to survey mutations in long-QT syndrome–related genes responsible for dLQTS in 20 patients who had been referred to our institutes consecutively over the past 11 years and analyze the functional effects induced by these mutations.

Expression Plasmids

The expression plasmids pRES2-EGFP/KCNQ1/wild type [WT]/KCNQ1 and pRe-CMV/KCNH2 [wild-type [WT]/KCNH2] were kindly provided by Dr J. Barhanin (UMR 6097 CNRS and Université de Nice Sophia Antipolis, Valbonne, France) and Dr M. Sanguinetti (University of Utah, Salt Lake City, Utah), respectively. The mutations were introduced using overlap PCR. The mutant plasmids were constructed by substituting the 857-bp XhoI-BglII for the 2313 and R243H mutants, 287-bp Eag1-BstEI for the D342V mutant, 794-bp BseEI-BglII for the H492Y mutant, or 392-bp BglII-Sphi fragments for S706F and M756V mutants, respectively, for the corresponding fragments of WT/KCNQ1 or WT/KCNH2.

Cell Transfection

Functional potassium channels were expressed transiently in Chinese hamster ovary (CHO) cells by transfecting the same amount of α subunit plasmids (1 μg/mL KCNQ1 cDNA or 2 μg/mL KCNH2 cDNA). For the analysis of IKs currents, the same amount of pRES/CD8-KCNE1 was coexpressed. Cells were trypsinized, diluted with Dulbecco Modified Eagle’s Medium (DMEM, Nacalai Tesque, Kyoto, Japan), and plated on 35-mm-diameter dishes containing 30 μL/mL penicillin, and 30 μg/mL streptomycin. The DMEM used for cell culture dishes was changed to OptiMEM (Invitrogen, Carlsbad, Calif) for transfection, and, after the addition of 10 μL lipofectamine (Invitrogen) and cDNA, the cells were incubated at 37°C for 3 hours, unless otherwise described. OptiMEM was then replaced by DMEM and the cells were subjected to electrophysiological measurements 48 to 72 hours after transfection. Cells expressing the potassium channels were selected through detection of green fluorescent protein and by decoration with anti-CD8 antibody-coated beads.

Electrophysiology

Whole-cell patch-clamp recordings were made at 37°C, using an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany) with pipettes filled with (in mM): 70 aspartate, 70 KOH, 40 KCl, 10 KH2PO4, 1 MgSO4, 3 Na2-ATP, 0.1 Li2-GTP, 5 HEPES, and 5 EGTA (pH 7.3 with 1N KOH), with a resistance of 2.0 to 4.0 mol/LΩ. The external superfusate contained (in mM): NaCl 140, KCl 5.4, MgCl2 0.5, CaCl2 1.8, NaH2PO4 0.33, glucose 5.5, and HEPES 5 (pH 7.4 with NaOH). Data were filtered at 2 kHz. Data acquisition was performed using PatchMaster acquisition software (HEKA). The holding potential was set at −80 mV. Current densities (pA/pF) were calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance. Current-voltage relations were fitted with the Boltzmann function:

\[ I/V_{1/2} = 1/(1 + \exp((V_1/2 - Vm)/k)) \]

where \( V_{1/2} \) indicates the potential at which the activation or inactivation is half-maximal, \( Vm \) the test potential, and \( k \) the slope factor.

Drug sensitivities were examined by various concentrations for erythromycin, disopyramide, and pirmenol (gift from Pfizer Inc, Groton, Conn). Depolarizing pulses were applied every 15 seconds and peak tail currents at −60 mV after +20 mV test potential were recorded in the absence or presence of various concentrations of agent. Percent inhibition was calculated by dividing the peak amplitude in the presence of drug by control. Drug concentration-inhibition relations were fitted to the Hill equation:

\[ \text{Fractional drug inhibition} = 1/(1 + (\text{IC}_{50}/[\text{drug}])^n) \]

where \( \text{IC}_{50} \) is the amount of drug necessary to produce the half-maximal inhibition of \( I_{Ks} \) tail currents, and \( n \) is the Hill coefficient for the fit.

Computer Simulation

The dynamic Luo-Rudy model (Clancy and Rudy 2001 model) of a ventricular cell was used, with recent modifications and action potentials were simulated using a previously reported model. The ratio of \( I_{Ks} \) and \( I_{Ks} \) conductance of M cell layer was set at 23.7. Based
on the experimental data of voltage-clamp recordings of KCNQ1 and KCNH2 channels heterologously expressed in CHO cells, we constructed Markov or Hodgkin-Huxley models for simulated mutant channels as compared with mutants associated with cLQTS (see supplemental data 1). To make the mutant channel models, we decreased the conductance of each channel as appropriate for the decreased current density and looked for adequate changes for mutant channels by changing each coefficient value, in turn, for gating states associated with impaired gating defects. The simulation for voltage-clamp experiments was calculated using the 4th-order Runge-Kutta method with a fixed-time step of 0.020 ms. The simulation programs (see supplemental data 1) were coded in C++ and implemented for personal computers.

**Statistical Analysis**

Experimental data are expressed as mean±SD and other clinical data as mean±SD, and the statistical comparisons were made using the unpaired Student t test. Differences in the positive mutation rate between 2 groups were analyzed by χ² and Fisher exact probability test. Statistical significance was considered as P<0.05.

**Results**

**Molecular Genetics of dLQTS**

Table 1 summarizes the clinical characteristics of 20 subjects with dLQTS (14 women and 6 men; mean age, 65±16 years). Nineteen subjects had TdP with marked QT prolongation, and 1 had syncope without documentedTdP after taking 1 of the drugs listed in Table 1. The average QTc interval before taking drugs, available for 15 subjects, was 446±29 ms. The QTc interval was significantly prolonged to 616±91 ms after taking 1 of the culprit drugs (versus QTc interval before taking drugs, P<0.001) and significantly shortened to 441±33 ms after washout of drugs (versus QTc interval after taking drugs, P<0.001). However, in 3 patients (cases 8, 13, and 18 in Table 1) a prolonged QT interval of over 480 ms was maintained after washout. The average R-R interval immediately before TdP was 1.2±0.4 seconds, and the average serum potassium level after TdP was 3.9±0.6 mEq/mL. In the majority of subjects (n=14, 70%), dLQTS was induced by antiarrhythmic drugs (disopyramide, pirmenol, cilenzolone, procainamide, and aminedrine in 8, 3, 1, 1, and 1 subjects, respectively; cases 1 to 14); the remaining cases were induced by antiarrhythmics, antibiotics, psychiatric, or miscellaneous drugs (cases 15 to 20 in Table 1). None had a family history of long-QT syndrome, whereas 3 subjects (cases 4, 14, and 20) had unexplained syncope and another subject (case 17) had a family member with sudden cardiac death. During a mean follow-up period of 52±44 months after discontinuing drugs, 1 subject without any gene mutations (case 9) had recurrent ventricular fibrillation. Compared with cLQTS, the age at first cardiac event in subjects diagnosed with dLQTS was significantly older (dLQTS versus cLQTS: 65±16 versus 19±18 years, P<0.001). No subject in the dLQTS group had a family member with long-QT syndrome (dLQTS versus cLQTS: 0% versus 24% subjects, P<0.001). The QTc interval in dLQTS subjects was significantly shorter than in cLQTS subjects (dLQTS versus cLQTS: 446±29 versus 507±71 ms, P<0.001); the Schwartz score was significantly lower in dLQTS than in cLQTS (dLQTS versus cLQTS: 0.9±1.4 versus 4.1±2.1, P<0.001).

In 8 (40%) dLQTS subjects, the genetic analysis identified 8 mutations in LQTS-related genes: 2 KCNQ1, 5 KCNH2, and 1 SCN5A (Table 1). These variants were not observed in the control subjects (220 chromosomes from non-cLQTS and non-dLQTS subjects), suggesting that they represented disease-related mutations. The 91 cLQTS patients also had gene mutations such as 33 KCNQ1, 36 KCNH2, 12 SCN5A, 2 KCNE2, and 8 compound mutations, and the positive mutation rate was similar between dLQTS and cLQTS subjects (8 of 20 [40%] versus 91 of 176 [52%] subjects, respectively, P=0.32, Figure 1A). These mutations were found in only 3 of 14 (21%) subjects with TdP induced by antiarrhythmic drugs. In contrast, 5 of 6 subjects with TdP induced by nonantiarrhythmic drugs had gene mutations (83%; versus patients with TdP induced by antiarrhythmic drugs, P<0.05) (Figure 1B). Seven of the 8 mutations were located in the nonpore regions (red circles in Figure 1C), except for the A614V-KCNH2 mutation in a case of hydroxyzine-induced TdP. The green circles in Figure 1C indicate the location of 8 mutations previously reported in dLQTS.5–10

**Clinical Characteristics of Genotyped dLQTS Subjects**

Detailed subject characteristics are presented in Table 1 and the supplemental data 2. Case 2 (M756V/KCNH2) was a 63-year-old man who was admitted with syncope after taking pirmenol (300 mg/d). Case 4 (H492Y/KCNH2) was a 52-year-old woman who had syncope while taking disopyramide (300 mg/d). She had previously had 1 episode of unexplained syncope. Case 14 (R243H/KCNQ1) was a 52-year-old woman who had repetitive syncope after taking aprindine (60 mg/d). Case 15 (S706F/KCNH2) was a 21-year-old woman who complained of sudden onset of palpitations and dyspnea after taking amphetamine and methamphetamine. An SCN5A mutation (L1825P/SCN5A) (case 16), has been reported previously; this concerned a 70-year-old woman who had TdP and prolongation of the QT interval after taking cisapride (5 mg/d) and pirmenol (200 mg/d). Case 17 (D342V/KCNH2) was a 70-year-old woman who had repetitive syncope due to erythromycin intake (1200 mg/d). This subject had a 24-year-old sister who had died suddenly, but it is not unknown if her sister had suffered from LQTS. Case 18 (R231C/KCNQ1) was a 72-year-old woman who had presyncope while taking probucol (250 mg/d). Case 20 concerned a 34-year-old woman (A614V/KCNH2 according to Table 1) who had syncope and QT prolongation induced by hydroxyzine (3 mg/d). None of the 20 subjects in the study had structural heart disease or a family history of documented LQTS. In genotyped families, a genetic test for 5 members revealed 1 R243H/KCNQ1 mutation carrier. This carrier had no syncope and a normal QT interval (QTc, 400 ms). The Schwartz scores in the 8 subjects with mutations were 1.0±1.5 points (range, 0 to 4 points). Thus, among the genotyped dLQTS subjects in this current study, there was only a low or moderate possibility of LQTS being present, both before taking the drugs or after their withdrawal.
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Ave ± SD | 65 ± 16 | 91 ± 6 | 33 ± 3 | 1.2 ± 0.4 |

M indicates male; F, female; NA, not available; AF, atrial fibrillation; Kent (+), type A Wolf-Parkinson-White syndrome; prolonged QT, ECG shows prolonged QT interval but unspecified QT interval; normal, normal QT interval but unspecified QT interval.

*intravenous administration.
Electrophysiological Characteristics of Mutations Associated With dLQTS

The biophysical effects of the respective KCNJ1 and KCNH2 mutations were analyzed using a heterologous expression system with the CHO cell line. The upper panel of Figure 2A shows representative current traces for WT and 2 mutant KCNJ1 channels; the lower panel shows those recorded from cells cotransfected with WT and each mutant. On their own, both mutants displayed smaller currents compared with WT, whereas the R231C channel was a slightly open K⁺/H⁺ leak channel. Under heterozygous conditions, however, they displayed currents comparable to those of WT without dominant negative effects.

At the end of the depolarizing pulse to 40 mV, current densities were smaller than those of WT channels (77.0 ± 10.6 pA/pF) in R231C/WT (39.6 ± 11.9 pA/pF, P < 0.05 versus WT) but not in R243H/WT (61.9 ± 10.7 pA/pF) (Figure 2B).

On the other hand, the R243H channels showed a significant positive shift of steady-state activation curve (Figure 2C). Half activation voltages (V½) and k were -8.2 ± 3.1 mV and 12.6 ± 0.6 for WT, -12.8 ± 3.6 mV and 13.6 ± 1.3 for R231C/WT, 1.7 ± 3.1 mV and 12.9 ± 0.6 for R243H/WT, respectively (V½: WT versus R243H/WT, P < 0.05). Figure 2D shows representative families of current traces (left panel) and time constants (right panel) of deactivation in each channel. The time course of deactivating kinetics could be fitted by a single-exponential function. The R243H/WT channel had faster deactivation process over -60 mV, whereas this process in the R231C/WT channel was slower than WT under -90 mV.

Figure 3A shows representative families of current traces recorded during depolarizing pulses from CHO cells transfected with KCNH2 cDNAs as indicated in the graph. The left column depicts current traces from cells transfected with each construct alone and the right column those recorded under heterozygous conditions except for the uppermost traces (WT g). When expressed alone, D342V was nonfunctional and 3 other mutations displayed functional channels. When coexpressed with WT, D342V showed weak dominant negative effects, whereas the other 3 channels showed nondominant negative effects. The functional outcome of the remaining mutation, A614V-KCNH2, has recently been reported. Using an oocyte expression system, Nakajima et al reported that the A614V channel showed loss of function in a dominant negative manner, and the results from the present study were almost identical (18% current density of WT).

Current densities at the end of a 2-second depolarization pulse were calculated in multiple cells and plotted as a function of test potential in Figure 3B. At the end of the depolarizing pulse to +20 mV, the densities were smaller than those of WT channels (36.2 ± 6.8 pA/pF) in D342V/WT (18.7 ± 4.5 pA/pF, P < 0.05 versus WT) and S706F/WT (21.9 ± 2.7 pA/pF, P = 0.055 versus WT). Those in...
H492Y/WT and M756V/WT were also smaller than in the WT, but the difference did not reach statistical significance.

The mean peak amplitudes of tail currents at various test potentials (2-second duration) plotted as a function of test potentials are displayed in Figure 3C. H492Y and M756V channels displayed amplitudes of peak tail currents similar to WT. For example, the peak tail current densities after a test pulse to 20 mV were 66.2 ± 10.5 pA/pF for WT, 62.1 ± 13.0 pA/pF for H492Y/WT, and 58.4 ± 7.7 pA/pF for M756V/WT. On the other hand, the
current densities of D342V/WT (1.5 ± 0.9 pA/pF, P < 0.001 versus WT) and S706F/WT (38.9 ± 7.3 pA/pF, P < 0.05 versus WT) channels were significantly smaller than WT. D342V/WT channel displayed currents smaller than WT (indicated by dotted line), whereas S706F/WT currents were similar to WT in size. Thus, D342V channels had weakly dominant negative suppression effects on reconstituted \( I_{Kr} \)-like currents, whereas S706F had no dominant negative suppression effect. To examine the voltage dependence for activation, Boltzmann function curves were fitted to the relationship between peak tail currents and test voltages under respective conditions and are represented by solid lines in Figure 3C. Half inactivation voltages \( (V_{1/2}) \) were 

\[
V_{1/2} = -23.2 ± 1.6 \text{ mV for WT,} 
-19.8 ± 3.0 \text{ mV for D342V/WT,} 
-24.6 ± 1.4 \text{ mV for H492Y/WT,} 
-26.1 ± 1.5 \text{ mV for S706F/WT,} 
\text{and} 
-19.8 ± 1.7 \text{ mV for M756V/WT, respectively (Figure 3D). Therefore, the mutations did not affect the voltage-dependent activation of the reconstituted } I_{Kr} \text{-like channel.}
\]

Whether the mutations affected the inactivation kinetics of \( KCNH2 \) channels was then assessed. Figure 4A shows the voltage dependence of availability of WT and mutant channels measured by a brief repolarization method (inset). With a 1-second depolarizing pulse, peak tail current amplitudes (arrow in the inset) after short preconditioning voltage pulses (5 ms) are plotted against the voltage of conditioning pulse. Mutant channels caused significant voltage shift of channel
availability to the hyperpolarizing direction compared with WT ($V_{1/2}$ of $-58.3 \pm 4.7$ mV for WT, $V_{1/2}$ of $-61.4 \pm 6.5$ mV for D342V/WT, $-77.8 \pm 4.7$ mV for H492Y/WT, $-70.1 \pm 3.2$ mV for S706F/WT, and $-71.1 \pm 4.6$ mV for M756V/WT, respectively). The time course to recovery from or the development of the inactivation (recovery from inactivation at hyperpolarized potentials and development of inactivation at $-70$ mV) was analyzed by double or triple pulse protocols. The time course of inactivating kinetics could be fitted by a single-exponential function. Time constants thus calculated were significantly smaller than WT and mutant channels over a wide range of voltage (between $-50$ and $+40$ mV for D342V/WT and H492Y/WT; between $-30$ and $+20$ mV for S706F/WT; between $-40$ and $0$ mV for M756V/WT, respectively). These results demonstrate that drug-induced LQTS mutants have accelerated inactivation kinetics. Figure 4C shows representative families of current traces (left panel) and time constants (right panel) of deactivation in each channel. When the time course of deactivating kinetics was fitted by a double-exponential function, the S706F/WT channel slightly accelerated the deactivation process (Figure 4C).

Most of the drugs that induced LQTS in the study subjects have been known to block $I_{Kr}$ in a concentration-dependent manner, whereas $I_{Kr}$ channel with KCNH2 mutations may have different drug sensitivities compared with the WT channel. Figure 5 shows 3 sets of drug concentration-current inhibition relationships associated with KCNH2 mutations with respect to erythromycin (5A), disopyramide (5B), and pirmenol (5C). In each case of drug-induced LQTS, an electrophysiological assay of current inhibition by the respective culprit drug was performed, for example, hydroxyzine, erythromycin, disopyramide, and pirmenol. IC$_{50}$S were not significantly different between WT and the respective mutant channels, suggesting that a change in drug sensitivity was not involved in causing the drug-induced TdP in the study subjects.
Computer Simulation of Ventricular Action Potentials

To compare how the functional changes caused by mutants affect ventricular action potentials, a simulation study was conducted using the Luo-Rudy computer model that incorporated the Markov16 or Hodgkin-Huxley23 process gating for the mutant channels (Figure 6A). Table 2 shows parameters of simulation that have been changed to fit to experimental results. First, the $I_{K_s}$ conductance was reduced to the level observed in the D342V/WT, A614V/WT, and R231C/WT channels. The deactivation time course for R231C/WT was also fitted by modifying a parameter. Second, the transition rate was changed accordingly from inactivation to open states ($\alpha_i$) and fitted with the experimental data seen in H492Y/WT and M756V/WT channels. Acceleration of inactivation induced by modifying the transition rate $\alpha_i$ reproduced smaller amplitudes for I-V relationships and negative shift of steady-state inactivation curve (Figure 4A); these findings were compatible with the experimental results (Figure 3, A through C). Third, some parameters were altered to simulate the S706F/WT model, by reducing the $I_{K_F}$ conductance, modifying $\alpha_i$, and increasing the transition rate from open to deactivation states. This was followed by change to parameters associated with the activation and deactivation rates for the R243H/WT model. Finally, not only was $I_{K_s}$ conductance decreased in the subjects, but the burst mode was also added to simulate the sustained current for the L1825P model.24 The L1825P/WT channel was heterogeneously simulated to equally mix WT and L1825P models.

In the simulated M cells using the Luo-Rudy model, the order of increase in magnitude of action potential duration (APD) was A614V/WT> R231C/WT> D342V/WT > S706F/WT > R243H/WT = L1825P/WT > H492Y/WT > M756V/WT for dLQTS mutations (Figure 6B, middle panel). Typically, for cLQTS mutations, the simulated APD was longer than for WT or drug-induced models, whereas APDs in drug-induced models were intermediate between those in WT and those in cLQTS (clinical information for simulated cLQTS are presented in supplemental Table 1).

Discussion

There were 3 major findings.1 In 8 of 20 consecutive dLQTS subjects, 5 KCNH2, 2 KCNQ1, and 1 SCN5A heterozygous missense mutations were identified; there was a similar positive mutation rate with dLQTS compared with cLQTS.2 Both KCNQ1 and KCNH2 mutants possessed loss of function effects on reconstituted $I_{K_s}$ or $I_{K_F}$-like channels.3 The functional changes in mutant channels reconstituted by the computer simulation resulted in a mildly prolonged APD, suggesting that the dLQTS may partially have a genetic background, especially mild or latent long-QT syndrome–associated mutations.

Mutations in dLQTS

Potential torsadegenic drugs are used in the clinical setting and include antiarrhythmic drugs, antibiotics, antihistamines, psychotropic drugs, and cholinergic antagonists. These might induce TdP, which may lead to the sudden cardiac death of individuals whose QT intervals were within normal range before taking the drug. Several drugs such as cisapride and terfenadine have been withdrawn from the market because of these possible side effects.25,26 The incidence of dLQTS is not high, and the drugs lead to TdP in only a small percentage of individuals, suggesting that there may be an underlying genetic background that predisposes these individuals to the risk.

Figure 5. Both WT and mutant channels showed similar drug sensitivities to the culprit agents. A, Fractional blockade by micromolar erythromycin was recorded with regard to D342V/WT or WT as described in the methods and is plotted against the drug concentrations. B, Similarly, the relationship between the fractional block by disopyramide and its concentration are shown. C, The relationship between the fractional pirmenol block of M756V/WT or WT channels and its concentration. IC50s for erythromycin, disopyramide, and pirmenol were 327 μmol/L (WT) and 248 μmol/L (D342V/WT), 13.9 μmol/L (WT) and 8.4 μmol/L (H492Y/WT), and 16.6 μmol/L (WT) and 13.4 μmol/L (M756V/WT), respectively (n=4 or 5 cells per condition).
In the medical literature, 13 KCNQ1 or KCNH2 mutations (13 of 15 mutations, 87%) associated with dLQTS, including 6 of the mutations identified in this present study, have been located in nonpore regions (Figure 1B). Mutations in non-transmembrane regions have been shown to cause either mild long-QT syndrome or benign clinical phenotypes. Mutation sites may influence the clinical and basic electrophysiological characteristics of patients with dLQTS. It is of interest that

Figure 6. Simulation study of congenital and dLQTS-associated channels. A, Scheme showing a Markov model for $I_{Kr}$ channels and simulation results for voltage-clamp protocols with a modified the transition rate $\alpha_i$: $\alpha_i = 0.439 \times \exp(-0.02352 \times (V + 40)) / K_{out}$. The 3 panels illustrate the result on the M756V/WT model, (a); the I-V relationship, (b); the I-V relationship for amplitudes of peak tail currents and (c); the steady-state inactivation curves. Open circles, WT; filled circles, mutant. B, Simulation study of action potential durations (APD). The parameters used for simulation were changed and matched the experimental results for voltage-clamp protocols. Myocardium models were stimulated at the cycle length of 600 ms for 5 minutes. C, Simulated APDs with $I_{Kr}$-blocking effects. When $I_{Kr}$ conductance was decreased to 11% in each of the models, the D342V model showed early afterdepolarization, whereas the WT model had only slight prolonged APD. Bold lines, controls; dotted lines, models with $I_{Kr}$-blocking effects.
Table 2. Parameters of Simulation Data in LQTS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
<th>Mutant</th>
<th>WT Basal Parameters</th>
<th>Mutant Changed Parameters</th>
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</thead>
<tbody>
<tr>
<td>dLQTS</td>
<td>KCNQ1</td>
<td>R231C</td>
<td>gks = 0.202*(1 + 0.6/((1 + pow(0.000038/cali), 1.4)))</td>
<td>gks = 0.103*(1 + 0.6/((1 + pow(0.000038/cali), 1.4)))</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>tauxs1 = 1/0.0000719*(v + 30)/(1 - exp(-0.148...))</td>
<td>tauxs1 = 1.1/0.0000719*(v + 30)/(1 - exp(-0.148...))</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ1</td>
<td>R243H</td>
<td>xs1ss = 1/(1 + exp(- (v - 1.5)/16.7))</td>
<td>xs1ss = 1/(1 + exp(- (v - 11.5)/16.7))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tauxs1 = 1/0.0000719*(v + 30)/(1 - exp(-0.148...))</td>
<td>tauxs1 = 0.9/0.0000719*(v + 30)/(1 - exp(-0.148...))</td>
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<tr>
<td>dLQTS</td>
<td>KCNH2</td>
<td>D342V</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0043*pow(Kout, 0.59)</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNH2</td>
<td>H492Y</td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 25))*4.5/Kout</td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 47))*4.5/Kout</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNH2</td>
<td>S706F</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0096*pow(Kout, 0.59)</td>
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<td></td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 25))*4.5/Kout</td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 39))*4.5/Kout</td>
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<tr>
<td>dLQTS</td>
<td>KCNH2</td>
<td>M756V</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0024*pow(Kout, 0.59)</td>
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<td>A614V</td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 25))*4.5/Kout</td>
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<td>cLQTS</td>
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<td>A341V</td>
<td>gks = 0.202*(1 + 0.6/((1 + pow(0.000038/cali), 1.4)))</td>
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<td>A344Aspl</td>
<td>gks = 0.202*(1 + 0.6/((1 + pow(0.000038/cali), 1.4)))</td>
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<td>KCNH2</td>
<td>A561V</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0023*pow(Kout, 0.59)</td>
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<tr>
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<td></td>
<td></td>
<td>αcil = 65.5e-3<em>exp(Exp(0.05574135</em>(v - 36))</td>
<td>αcil = 65.5e-3<em>exp(Exp(0.05574135</em>(v - 16))</td>
</tr>
<tr>
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<td>KCNH2</td>
<td>G604S</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0040*pow(Kout, 0.59)</td>
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<td></td>
<td></td>
<td></td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 25))*4.5/Kout</td>
<td>αcil = 0.439<em>exp(-0.02352</em>(v + 35))*4.5/Kout</td>
</tr>
<tr>
<td>cLQTS</td>
<td>KCNH2</td>
<td>A614V</td>
<td>gherg = 0.0135*pow(Kout, 0.59)</td>
<td>gherg = 0.0024*pow(Kout, 0.59)</td>
</tr>
<tr>
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<td>L619F</td>
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<td>Gna = 6.24</td>
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<td>No burst mode</td>
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<td></td>
<td>αcil = 6e-6</td>
<td>βcil = 3e-4</td>
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<td>Gna = 6.24</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>αcil = 6e-6</td>
<td>βcil = 3e-4</td>
</tr>
</tbody>
</table>

The functional assay of our 7 mutations resulted in various levels of loss of function, but most of them showed no dominant negative suppression, which is usually observed in the classic clQTS. Clinical characteristics during the drug intake were not significantly distinct from those of clQTS, which shares a similar genetic background with the dlQTS. Several polymorphisms have been shown to be associated with dlQTS.4,28 Abbott et al4 identified a polymorphism (T8A) of the KCNE2 gene encoding MiRP, a β-subunit for the IKr channel, which is present in 1.6% of the population and is associated with TdP induced by quinidine or sulfamethoxazole/trimethoprim administration. Saplinski et al28 also found a heterozygous polymorphism involving substitution of serine with tyrosine (S1102Y) in the sodium channel gene SCN5A among blacks that increased the risk for drug-induced TdP. The polymorphism was present in 57% of 23 patients with proarhythmic episodes but in only 13% of control subjects. These findings suggested that common genetic variations may increase the risk for development of drug-related arrhythmias.

Roden et al29 reported that ciparide could rescue trafficking of L1825P channel with a potentially sustained current and revealed a new mechanism of dlQTS with the Luo-Rudy model. However, in the genotyped subjects in the present study, dlQTS mainly resulted from the IKr-blocking effect of culprit drugs in the presence of latent genetic backgrounds. Cardiac repolarization reserve may protect subjects against drug-induced TdP. In the presence of latent genetic backgrounds, however, reduction in the repolarization reserve unveils the presence of so-called “concealed” long-QT syndrome when drugs with IKr-blocking effects are administered.30,31 In the presence of latent genetic backgrounds, however, reduction in the repolarization reserve unveils the presence of so-called “concealed” long-QT syndrome when drugs with IKr-blocking effects are administered.30,31 The presence of borderline prolongation of the QT interval, together with personal information such as unexplained previous syncope and family history of premature sudden death, may help to prevent drug-induced arrhythmia even if a subject’s Schwartz score is low, because they could have a potential risk of TdP. Special attention should be paid to family members of the index subject with drug-induced QT prolongation because 30% of family members were found to have a predisposing genetic background in the present study. Indeed, they may have inherited the risk for being susceptible to dlQTS.

Limitations of the Study
This study has some limitations. Because of the small cohort of dlQTS subjects, this study was not powered to quantify the overall prevalence of ion channel mutations in the group of subjects with drug-induced TdP; there was also a possible...
selection bias in the population. For the causative agents, we were unable to test the action of amphetamine and methamphetamine because it was impossible to obtain these illegal drugs for clinical study. However, a previous report has shown that 3,4-methylenedioxy methamphetamine (ecstasy, NMDA) prolongs the APD of hippocampal neurons by blocking the conductance of a resting K⁺ channel.32 It is quite possible that these drugs also suppressed cardiac K⁺ currents and induced QT prolongation and TdP in 1 subject in our study, based on her medical records. Regarding protein trafficking, 2 mutations in this study, A614V in SCN5A, L1825P in KCNH2 and M756V in KCNH2, would be not trafficking-deficient because, under heterozygous conditions, these mutants showed adequate current density compared with WT. In the simulation study, the parameter settings could mimic mutant channels. In addition, the setting of the parameters might have innumerable patterns, and it therefore remains possible that other combinations of patterns could also simulate mutant channels.

Acknowledgments
We thank Arisa Ikeda for excellent technical assistance.

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Disclosures
None

References
SCN5A sodium channel implicated in risk of cardiac arrhythmia. Science. 2002;297:1333–1336.


CLINICAL PERSPECTIVE

Drug-induced long-QT syndrome (dLQTS) is a disease associated with the appearance of a prolonged QT interval and torsades de pointes after taking a culprit drug or drugs; the QT interval usually returns to within normal range after a washout period of these drugs. The clinical phenotype of dLQTS that appears during administration of these drugs resembles that of congenital long-QT syndrome (cLQTS), and “latent” genetic factors may underlie the susceptibility of a subject to drug-induced serious adverse reactions, such as a long QT interval and torsades de pointes. In the analysis of cLQTS-associated genes encoding cardiac ion channel-composing proteins, this study revealed that dLQTS had a similar positive mutation rate compared with cLQTS. When reconstituted in Chinese hamster ovary cells, KCNQ1 and KCNH2 mutant channels showed complex gating defects without dominant negative effects or a relatively mild decreased current density. With the Luo-Rudy simulation model of action potentials, action potential durations of most mutant channels were between those of wild-type and cLQTS. In conclusion, although the dLQTS subjects had genetic backgrounds that were similar to cLQTS subjects, the functional changes associated with these mutations identified in dLQTS were different from those in cLQTS. Thus, we believe that dLQTS can be regarded as a latent form of long-QT syndrome. When $I_{Kr}$-blocking agents produce excessive QT prolongation, the underlying genetic background of the dLQTS subject should be taken into consideration, as would be the case with cLQTS.
Latent Genetic Backgrounds and Molecular Pathogenesis in Drug-Induced Long-QT Syndrome

Hideki Itoh, Tomoko Sakaguchi, Wei-Guang Ding, Eiichi Watanabe, Ichiro Watanabe, Yukiko Nishio, Takeru Makiyama, Seiko Ohno, Masaharu Akao, Yukei Higashi, Naoko Zenda, Tomoki Kubota, Chikara Mori, Katsunori Okajima, Tetsuya Haruna, Akashi Miyamoto, Mihoko Kawamura, Katsuya Ishida, Iori Nagaoka, Yuko Oka, Yuko Nakazawa, Takenori Yao, Hikari Jo, Yoshihisa Sugimoto, Takashi Ashihara, Hideki Hayashi, Makoto Ito, Keiji Imoto, Hiroshi Matsuura and Minoru Horie

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SUPPLEMENTAL MATERIAL
Supplemental data 1.

Header Files (LRc.h)

//

#define WINDOWS 1

#define DISPLAY 1 // 0: no display, 1: display trace (only for Windows)
#define DATA_FILE 1 // 0: No file output, 1: Text file, 2: Binary file
#define NTRACES 1

#define NUMBER_OF.Cells 1 // Number of cells

#define TIMESTEP 0.002 // in ms
#define BEATS 500 // Number of beats
#define BCL 600 // Basic Cycle Length (ms)

#define STIM -120 // Stimulation intensity

#define MARKOV_NA 0 // 0: conventional HH-model, 1: Markov model

#define MARKOV_HERG 1 // 0: conventional HH-model, 1: Markov model

#define WT_NA_CHANNEL_G (16.0 * 0) //WT_NA_CHANNEL_G (8.0 * 1)
#define MT_NA_CHANNEL_G (16.0 * 0)

//-------------------------------

// include files

#include <stdlib.h>
#include <stdio.h>
#include <fcntl.h>
#ifndef WINDOWS
#include <process.h>
#endif
#include <io.h>
#include <string.h>
#include <math.h>

#include “MarkovNaChannel.h”
#include “MarkovHERGChannel.h”
#include “LRModel.h”
// LRd Model Code
// The Luo–Rudy Dynamic (LRd) Model of the Mammalian Ventricular Myocyte
// Gregory Faber
// This code requires a C++ compiler
// Detailed list of equations and model description are provided in
// Circ Res 1995;77:140-152
// Biophys J 1995;68:949-966
// Circulation 1999;99:2466-2474
// Cardiovas Res 1999;42:530-542
// Nature 1999;400:566-569
// Circulation 2000;101:1192-1198
// Biophy J 2000;78:2392-2404

class CMarkovNaChannel;

class CMyocyte
{
public:
    CMyocyte();
    ~CMyocyte();

public:
    void setStepTime(double ut);
    bool checkChanges(double t);
    void calculateChanges(double t);
    bool advanceStep(double t);

public:
    CMyocyte* pCellArray;
    int cellIndex;
    int nCells;
    double v; // Membrane voltage (mV)
    double bcl;
    double st; // Constant Stimulus (uA/cm^2)
    // double cai; // Intracellular Ca Concentration (mM)
    double irelcicr; // Ca release from JSR to myo. due to CICR (mM/ms)

    double ikr;
    double ikr2;

    double dvdtmax;
#if MARKOV_NA == 1
CMarkovNaChannel wtNaChannel;
CMarkovNaChannel mutantNaChannel;
#endif

#if MARKOV_HERG == 1
CMarkovHERGChannel HERGChannel;
#endif

private: // constant values
double R: // 8314: // Universal Gas Constant (J/kmol*K)
double frdy: // 96485: // Faraday's Constant (C/mol)
double temp: // 310: // Temperature (K)
double pi: // 3.141592: // Pi

// Ion Valences
double zna: // 1: // Na valence
double zk: // 1: // K valence
double zca: // 2: // Ca valence

private:
CMyocyte* getPrevCell(int ci);
CMyocyte* getNextCell(int ci):

// Ion Current Functions
void comp_ina (): // Calculates Fast Na Current
void comp_ical (): // Calculates Currents through L-Type Ca Channel
void comp_icat (): // Calculates Currents through T-Type Ca Channel
void comp_ikr (): // Calculates Rapidly Activating K Current
void comp_iks (): // Calculates Slowly Activating K Current
void comp_iki (): // Calculates Time-Independent K Current
void comp_ikp (): // Calculates Plateau K Current
void comp_ikna (): // Calculates Na-activated K Current
void comp_ikatp (): // Calculates ATP-Sensitive K Current
void comp_ito (): // Calculates Transient Outward Current
void comp_inaca (): // Calculates Na-Ca Exchanger Current
void comp_inak (): // Calculates Na-K Pump Current
void comp_insca (): // Calculates Non-Specific Ca-Activated Current
void comp_ipcaw (): // Calculates Sarcolemmal Ca Pump Current
void comp_icab (): // Calculates Ca Background Current
void comp_inab (): // Calculates Na Background Current
void comp_gap(): // Calculates gap-junction current
void comp_it (double t): // Calculates Total Current

// Ion Concentration Functions
void conc_nai (): // Calculates myoplasmic Na ion concentration
void conc_iki (): // Calculates myoplasmic K ion concentration
void conc_nsr (): // Calculates NSR Ca ion concentration
void conc_jsr (): // Calculates JSR Ca ion concentration
```cpp
void calc_itr();  // Calculates Translocation of Ca from NSR to JSR
void conc_cai();  // Calculates new myoplasmic Ca ion concentration
void conc_cleft();  // Calculates new cleft ion concentrations

private:
    // Time Step
    double dt;  // Time step (ms), used for calculating current changes
    double udt;  // Universal Time Step, used for calculating the voltage change
    int utsc;  // Universal Time Step Counter
    int nxstep;  // Interval Between Calculating Ion Currents
    //    int steps;      // Number of Steps
    //    int increment;  // Loop Control Variable
    //    int stimCounter;  // Stimulus Counter
    int printdata;
    int printval;
    bool printFlag;

    // cell geometory
    double l;  // = 0.01;       // Length of the cell (cm)
    double a;  // = 0.0011;     // Radius of the cell (cm)
    double vcell;  // Cell volume (uL)
    double ageo;  // Geometric membrane area (cm^2)
    double acap;  // Capacitive membrane area (cm^2)
    double vmoyo;  // Myoplasm volume (uL)
    double vmito;  // Mitochondria volume (uL)
    double vsr;  // SR volume (uL)
    double vnsr;  // NSR volume (uL)
    double vjsr;  // JSR volume (uL)
    double vcleft;  // Cleft volume (uL)

    // Total Current and Stimulus
    //    double st;       // Constant Stimulus (uA/cm^2)
    double tstim;  // Time Stimulus is Applied (ms)
    double stimtime;  // Time period during which stimulus is applied (ms)
    double it;  // Total current (uA/cm^2)

    // Voltage
    //    double v;       // Membrane voltage (mV)
    //    double vn;    // New Voltage (mV)
    double dvdt;  // Change in Voltage / Change in Time (mV/ms)
    bool triggerCICR;  // triggerCICR condition to test for dvdtmax
    //    double dvdtnew; // New dv/dt (mV/ms)

public:

    // Ion Concentrations
    double nai;  // Intracellular Na Concentration (mM)
    double nao;  // Extracellular Na Concentration (mM)
    double nabm;  // Bulk Medium Na Concentration (mM)
```

double dnao;  // Change in Cleft Na Concentration (mM)
double ki;    // Intracellular K Concentration (mM)
double ko;    // Extracellular K Concentration (mM)
double kbm;   // Bulk Medium K Concentration (mM)
double dko;   // Change in Cleft K Concentration (mM)
double cai;   // Intracellular Ca Concentration (mM)
double cao;   // Extracellular Ca Concentration (mM)
double cabm;  // Bulk Medium Ca Concentration (mM)
double dcao;  // Change in Cleft Ca Concentration (mM)
double cmdn;  // Calmodulin Buffered Ca Concentration (mM)
double trpn;  // Troponin Buffered Ca Concentration (mM)
double nsr;   // NSR Ca Concentration (mM)
double jsr;   // JSR Ca Concentration (mM)
double csqn;  // Calsequestrin Buffered Ca Concentration (mM)
taudiff; = 1000; // Diffusion Constant for Ion Movement from Bulk Medium to Cleft Space

  // Myoplasmic Na Ion Concentration Changes
  double naiont; // Total Na Ion Flow (uA/uF)
  double dnai;  // Change in Intracellular Na Concentration (mM)

  // Myoplasmic K Ion Concentration Changes
  double kiont; // Total K Ion Flow (uA/uF)
  double dki;  // Change in Intracellular K Concentration (mM)

  // NSR Ca Ion Concentration Changes
  double dnsr;  // Change in [Ca] in the NSR (mM)
  double iup;  // Ca uptake from myo. to NSR (mM/ms)
  double ileak; // Ca leakage from NSR to myo. (mM/ms)
  double kleak; // Rate constant of Ca leakage from NSR (ms^{-1})
  double kmup; = 0.00092; // Half-saturation concentration of iup (mM)
  double iupbar; = 0.00875; // Max. current through iup channel (mM/ms)
  double nsrbar; = 15; // Max. [Ca] in NSR (mM)

  // JSR Ca Ion Concentration Changes
  double djsr;  // Change in [Ca] in the JSR (mM)
  double tauon; = 2; // Time constant of activation of Ca release from JSR (ms)
  double tauoff; = 2; // Time constant of deactivation of Ca release from JSR (ms)
  double tcicr; // t=0 at time of CICR (ms)
  double irelcicr; // Ca release from JSR to myo. due to CICR (mM/ms)
  double csqnth; = 8.75; // Threshold for release of Ca from CSQN due to JSR overload (mM)
  double gmaxrel; = 150; // Max. rate constant of Ca release from JSR due to overload (ms^{-1})
  double grelbarjsrol; // Rate constant of Ca release from JSR due to overload (mM/ms)
  double greljsrol; // Rate constant of Ca release from JSR due to CICR (ms^{-1})
  double tjsrol; // t=0 at time of JSR overload (ms)
  double ireljsrol; // Ca release from JSR to myo. due to JSR overload (mM/ms)
  double csqnbar; = 10; // Max. [Ca] buffered in CSQN (mM)
  double kmcsqn; = 0.8; // Equilibrium constant of buffering for CSQN (mM)
  double bjsr; // b Variable for analytical computation of [Ca] in JSR (mM)
  double cjsr; // c Variable for analytical computation of [Ca] in JSR (mM)
double RyRopen; // Time constant of activation of Ca release from JSR (ms)
double RyRclose; // Time constant of deactivation of Ca release from JSR (ms)

double grel; // Magnitude of Ca release
double dcaiont; // Rate of change of Ca entry
double dcaiontnew; // New rate of change of Ca entry
double caiontold; // Old rate of change of Ca entry

// Translocation of Ca Ions from NSR to JSR

double itr; // Translocation current of Ca ions from NSR to JSR (mM/ms)
double taur:// = 180; // Time constant of Ca transfer from NSR to JSR (ms)

// Myoplasmic Ca Ion Concentration Changes

double caiont; // Total Ca Ion Flow (nA/uF)
double dcai; // Change in myoplasmic Ca concentration (mM)
double catotal; // Total myoplasmic Ca concentration (mM)
double bmymo; // b Variable for analytical computation of [Ca] in myoplasm (mM)
double cmymo; // c Variable for analytical computation of [Ca] in myoplasm (mM)
double dmyymo; // d Variable for analytical computation of [Ca] in myoplasm (mM)
double gpiig; // Tribute to all the guinea pigs killed for the advancement of knowledge

double cmdnbar:// = 0.050; // Max. [Ca] buffered in CMDN (mM)
double trpnbarn:// = 0.070; // Max. [Ca] buffered in TRPN (mM)
double kmcmdn:// = 0.00238; // Equilibrium constant of buffering for CMDN (mM)
double kmtrpn:// = 0.0005; // Equilibrium constant of buffering for TRPN (mM)

// Fast Sodium Current (time dependant)

double ina; // Fast Na Current (nA/uF)
double gna; // Max. Conductance of the Na Channel (mS/uF)
double ena; // Reversal Potential of Na (mV)
double am; // Na alpha-m rate constant (ms^-1)
double bm; // Na beta-m rate constant (ms^-1)
double ah; // Na alpha-h rate constant (ms^-1)
double bh; // Na beta-h rate constant (ms^-1)
double aj; // Na alpha-j rate constant (ms^-1)
double bj; // Na beta-j rate constant (ms^-1)
double m; // Na activation
double h; // Na inactivation
double j; // Na inactivation

// Current through L-type Ca Channel

double ilca; // Ca current through L-type Ca channel (nA/uF)
double ilcana; // Na current through L-type Ca channel (nA/uF)
double ilcak; // K current through L-type Ca channel (nA/uF)
double ilcatot; // Total current through the L-type Ca channel (nA/uF)
double ibarca; // Max. Ca current through Ca channel (nA/uF)
double ibarna; // Max. Na current through Ca channel (nA/uF)
double ibark; // Max. K current through Ca channel (nA/uF)
double d; // Voltage dependant activation gate
double dss; // Steady-state value of activation gate d
double taud;  // Time constant of gate d (ms^-1)
double f;  // Voltage dependant inactivation gate
double fss;  // Steady-state value of inactivation gate f
double tauf;  // Time constant of gate f (ms^-1)
double fca;  // Ca dependant inactivation gate
double kmca;  // = 0.0006:  // Half-saturation concentration of Ca channel (mM)
double pca;  // = 0.00054:  // Perimibility of membrane to Ca (cm/s)
double gacai;  // = 1:  // Activity coefficient of Ca
double gaca;  // = 0.341:  // Activity coefficient of Ca
double pna;  // = 0.000000675:  // Perimibility of membrane to Na (cm/s)
double ganai;  // = 0.75:  // Activity coefficient of Na
double pk;  // = 0.000000193:  // Perimibility of membrane to K (cm/s)
double gaki;  // = 0.75:  // Activity coefficient of K
double gako;  // = 0.75:  // Activity coefficient of K

double icat;  // Ca current through T-type Ca channel (uA/uF)
double gcat;  // Max. Conductance of the T-type Ca channel (mS/uF)
double eca;  // Reversal Potential of the T-type Ca channel (mV)
double b;  // Voltage dependant activation gate

double xrss;  // Steady-state value of inactivation gate xr

double xrs1ss;  // Steady-state value of inactivation gate xrs1

double xrs2ss;  // Steady-state value of inactivation gate xrs2

double prnak;  // = 0.01833:  // Na/K Permiability Ratio

double iki;  // Time-independant K current (uA/uF)
// Channel Conductance of Time Independent K Current (mS/uF)
double gki;

// Reversal Potential of Time Independent K Current (mV)
double ek;

// K alpha-ki rate constant (ms⁻¹)
double aki;

// K beta-ki rate constant (ms⁻¹)
double bki;

// K inactivation
double kin;

// Plateau Potassium Current
double ikp;

// Plateau K current (uA/uF)
double gkp;

// Channel Conductance of Plateau K Current (mS/uF)
double ekp;

// Reversal Potential of Plateau K Current (mV)
double kp;

// K plateau factor

// Na-Activated K Channel
double ikna;

// Na activated K channel
double pona;

// Open probability dependant on Na
double pov;

// Open probability dependant on Voltage

double ekna;

// Reversal potential

double gkna; // = 0.12848; // Maximum conductance (mS/uF)

// Hill coefficient for Na dependance

double nkna; // = 2.8;

// Hill coefficient for Na dependence

double kdna; // = 66; // Dissociation constant for Na dependence (mM)

// ATP-Sensitive K Channel

double ikatp; // ATP-sensitive K current (uA/uF)

double ekatp; // K reversal potential (mV)

double gkbaratp; // Conductance of the ATP-sensitive K channel (mS/uF)

double kpatp; // Maximum conductance of the ATP-sensitive K channel (mS/uF)

// Percentage availability of open channels

double natp; // = 0.24; // K dependence of ATP-sensitive K current

double nicholsarea; // = 0.00005; // Nichol's area (cm²)

double atpi; // = 3; // Intracellular ATP concentration (mM)

double hatp; // = 2; // Hill coefficient

double katp; // = 0.250; // Half-maximal saturation point of ATP-sensitive K current (mM)


double ito; // Transient outward current

double gitodv; // Maximum conductance of Ito

double ekdv; // Reversal Potential of Ito

double rvdv; // Time independent voltage dependence of Ito

double zdv; // Ito activation

double azdv; // Ito alpha-z rate constant

double bzdv; // Ito beta-z rate constant

// Time constant of z gate

double tauzdv;

double zssdv; // Steady-state value of z gate

double ydv; // Ito inactivation

double aydv; // Ito alpha-y rate constant

double bydv; // Ito beta-y rate constant

// Time constant of y gate

double tauydv;

double yssdv; // Steady-state value of y gate
// Sodium-Calcium Exchanger V-S
double inaca; // NaCa exchanger current (uA/uF)
double c1: /= 0.00025; // Scaling factor for inaca (uA/uF)
double c2: /= 0.00001; // Half-saturation concentration of NaCa exchanger (mM)
double gammas: /= .15; // Position of energy barrier controlling voltage dependence of inaca

// Sodium-Potassium Pump
double inak; // NaK pump current (uA/uF)
double fnak; // Voltage-dependance parameter of inak
double sigma; // [Na] dependence factor of fnak
double ibarnak: /= 2.25; // Max. current through Na-K pump (uA/uF)
double kmnai: /= 10; // Half-saturation concentration of NaK pump (mM)
double kmko: /= 1.5; // Half-saturation concentration of NaK pump (mM)

// Nonspecific Ca-activated Current
double insna; // Non-specific Na current (uA/uF)
double insk; // Non-specific K current (uA/uF)
double ibarnsna; // Max. Na current through NSCa channel (uA/uF)
double ibarnsk: // Max. K current through NSCa channel (uA/uF)
double pnsca: /= 0.000000175; // Permeability of channel to Na and K (cm/s)
double kmnsca: /= 0.0012; // Half-saturation concentration of NSCa channel (mM)

// Sarcolemmal Ca Pump
double ipca; // Sarcolemmal Ca pump current (uA/uF)
double ibarpca: /= 1.15; // Max. Ca current through sarcolemmal Ca pump (uA/uF)
double kmpca: /= 0.0005; // Half-saturation concentration of sarcolemmal Ca pump (mM)

// Ca Background Current
double icab; // Ca background current (uA/uF)
double gcab; // Max. conductance of Ca background (mS/uF)
double ecab; // Nernst potential for Ca (mV)

// Na Background Current
double inab; // Na background current (uA/uF)
double gnab; // Max. conductance of Na background (mS/uF)
double enab; // Nernst potential for Na (mV)

// gap junction current
double igap;
double ggap;
};
Header Files (MarkovHERGChannel.h)

//
//   Na channel Markov process model
//
//          IC3 - IC2 - IF - IM1 - IM2
//                |         | /   |
//                C3 - C2 - C1 - 0
//
//
//   xd[0]  C3  
//   xd[1]  C2  
//   xd[2]  C1  
//   xd[3]  0   
//   xd[4]  IC3 
//   xd[5]  IC2 
//   xd[6]  IF  
//   xd[7]  IM1 
//   xd[8]  IM2 
//
// integration methods
//   modified from Colleen Clancy's method
//

#define HERG_CHANNEL_PARAM_NUM 4
#define MARKOV_HERG_C3   0
#define MARKOV_HERG_C2   1
#define MARKOV_HERG_C1   2
#define MARKOV_HERG_O    3
#define MARKOV_HERG_I    4
#define OPEN_STATE       3
#define NUM_OF_HERG_STATES 5

class CMarkovHERGChannel
{

public:
   CMarkovHERGChannel();
   ~CMarkovHERGChannel();

public:
   double compCurrent(double t, double dt, double v, double ena, double ko);
private:
    void getdy(double dt, double* dy);
    void getRateConstants(double v);

public:
    double gherg;
    double pHERG[NUM_OF_HERG_STATES];

private:
    int ix, i4;
    int nStates;
    bool done;

double sum;
double Kout;
double aa, ain, a, b, bin, bb, ai, bi, mu;
double pC3, pC2, pC1;
double p0;
double pI;
double y[NUM_OF_HERG_STATES];
double yn[NUM_OF_HERG_STATES];
double k[NUM_OF_HERG_STATES];
double dny[NUM_OF_HERG_STATES];
double dnyl[NUM_OF_HERG_STATES];
};
Header Files (MarkovNaChannel.h)

// Na channel Markov process model
//
//    IC3 - IC2 - IF - IM1 - IM2
//        |   |   /   |
//        C3 - C2 - C1 - 0
//
//
//  xd[0]  C3
//  xd[1]  C2
//  xd[2]  C1
//  xd[3]  0
//  xd[4]  IC3
//  xd[5]  IC2
//  xd[6]  IF
//  xd[7]  IM1
//  xd[8]  IM2
//
// integration methods
// modified from Colleen Clancy's method
//

#define OPEN_STATE 3
#define OPEN_STATE_B 12
#define NUM_OF_NA_STATES 9
#define NUM_OF_NA_BURST_STATES 4

class CMarkovNaChannel
{
    public:
        CMarkovNaChannel();
        ~CMarkovNaChannel();

        double compCurrent(double t, double dt, double v, double ena);

    private:
        void getdy(double dt, double* dy);
        void getRateConstants(double v);
public:
    bool bBurstMode;
    double gna;
    double pNa[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];

private:
    int ix, i4;
    int nStates;
    bool done;

    double sum;
    double a11, a12, a13, a2, a3, a4, a5;
    double b11, b12, b13, b2, b3, b4, b5;
    double a6, b6;
    double y[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double yn[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double k[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double dny[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double dny1[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
};
Source Files (LRc.cpp)

//
// Luo-Rudy model
// + markov Na channel
// + multi-cell option
//
// Keiji Imoto
// Department of Information Physiology
// National Institute for Physiological Sciences
// Okazaki 444-8585, Japan
//
// Version
// 11-Dec-2003
// 09-Jan-2004 Clean up
// 10-Jan-2004 CICR trigger was enabled in non-stimulated cells
//
#include “LRc.h”

void flashData(double t, int nFileCounter, int stepCounter, double **a, int nTraces);

int main (int argc, char** argv)
{
    int i,j;

    int bcl = BCL;
    int beats = BEATS;
    int nCells = NUMBER_OF_CELLS;
    int nTraces = NTRACES;
    int steps, stepCounter;

    double udt = TIMESTEP; // time (ms)
    double t; // time (ms)
    bool checkFlag;

    int nFileCounter = 0;
    int nFileDataSize = 20*1024; // 100K

    #if MARKOV_NA == 1
        printf("Markov Na channel model\n");
    #else
        printf("Markov Na channel model\n");
    #endif
#ifdef
// text file
printf("Ascii output file\n");
#else
// binary file
printf("Binary output file\n");
#endif
printf("%d cells, BCL = %d, BEATS = %d\n", nCells, bcl, beats);
steps = (int) ((bcl * beats) / udt); // Number of steps.

// initialization of cells
CMyocyte* pCells = new CMyocyte[nCells];

for (j = 0; j < nCells; j++) {
pCells[j].pCellArray = pCells;
pCells[j].cellIndex = j;
pCells[j].nCells = nCells;
pCells[j].bcl = BCL;
pCells[j].setStepTime (udt);
#if MARKOV_NA == 1
pCells[j].wtNaChannel.gna = WT_NA_CHANNEL_G; //16.0 * 0.5;
pCells[j].wtNaChannel.bBurstMode = false;
pCells[j].mutantNaChannel.gna = MT_NA_CHANNEL_G; // 16.0 * 0.5;
pCells[j].mutantNaChannel.bBurstMode = true;
#endif
}
pCells[0].st = STIM;

// trace buffer
double** a = new double *[nTraces+1];
for (i = 0; i < nTraces+1; i++) a[i] = new double[nFileDataSize];

// main loop
t = 0;
stepCounter=0;
for (i = 0; i < steps; i++) {
t = i * udt;
// printf("t=%g\n", t);
for (j = 0; j < nCells; j++) {
    if (pCells[j].checkChanges (t)) {
pCells[j].calculateChanges (t);
}
checkFlag = false;
for (j = 0; j < nCells; j++) {
    if (pCells[j].advanceStep (t)) {
        checkFlag = true;
    }
}
if (checkFlag) {
    a[0][stepCounter] = t;
    for (j = 0; j < nCells; j++)a[j+1][stepCounter] = pCells[j].v;
    a[1][stepCounter] = pCells[0].v;
    //a[2][stepCounter] = pCells[0].fca; //fca
    //a[3][stepCounter] = pCells[0].cri;
    //a[4][stepCounter] = pCells[0].ina;
    //a[5][stepCounter] = pCells[0].jir;
    //a[6][stepCounter] = pCells[0].iks;
    //a[7][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C3];
    //a[8][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C2];
    //a[9][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C1];
    //a[10][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_I];
    //a[11][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_O];
    stepCounter++;
    if (stepCounter >= nFileDataSize){
        flashData(t, nFileCounter, stepCounter, a, nTraces);
        nFileCounter++;
        stepCounter=0;
    }
}
}    // end of main loop
flashData(t, nFileCounter, stepCounter, a, nTraces):

/*
      //--------Data output ----------------------

#if DATA_FILE == 1  // text file
    FILE* fap;
    char fname[]="ap.txt";
    printf("Ascii output file\n");
    fap = fopen(fname,"w");
    for(i=0;i<stepCounter;i++){
        fprintf(fap,"%lf", a[0][i]);
    }
*/
for (j=0; j<nTraces; j++) fprintf(fap, "%.1f", a[j+1][i]);
    fprintf(fap, "\n");
}
close(fap);
#elif DATA_FILE == 2  // binary file
FILE* fap;
    char fname[]="ap.dat";
    printf("Binary output file\n");
    fap = fopen(fname, "wb");
    double*ax = new double[nTraces+1];
    for (i=0; i<stepCounter; i++) {  
        for (j=0; j<nTraces+1; j++) ax[j] = a[j][i];
        fwrite(ax, sizeof(double), nTraces+1, fap);
    }
close(fap);
#endif

*/

    // clean up
    delete [] pCells;
    for (j=0; j<nCells+1; j++) delete [] a[j];
    delete [] a;

#if DISPLAY == 1
    char fname[] = "ap001.dat";
    char s[10];
    sprintf(s, "%d", nTraces);
    _spawnl (_P_NOWAIT, "gc", "gc", fname, s, NULL);
#endif

    return 0;
}

//--------Data output --------------------------

void flashData(double t, int nFileCounter, int stepCounter, double **a, int nTraces) {
    FILE *fap;
    int i, j;
    char fname[32]:

    nFileCounter++;
#if DATA_FILE == 1  // text file
    sprintf(fname, "ap%03d.txt", nFileCounter);
    fap = fopen(fname, "w");
    for (i=0; i<stepCounter; i++) {
        fprintf(fap, "%lf", a[0][i]);
        for (j=0; j<nTraces; j++) fprintf(fap, "\t%lf", a[j+1][i]);
        fprintf(fap, "\n");
    }
#endif

#elif DATA_FILE == 2  // binary file
    sprintf(fname, "ap%03d.dat", nFileCounter);
    fap = fopen(fname, "wb");
    double*ax = new double[nTraces+1];
    for (i=0; i<stepCounter; i++) {
        for (j=0; j<nTraces+1; j++) ax[j] = a[j][i];
        fwrite(ax, sizeof(double), nTraces+1, fap);
    }
#endif

fclose(fap);
    printf("t=%lf: %d points -> file <%s>\n", t, stepCounter, fname);

return;
}
Source Files (LRModel.cpp)

// Luo-Roudy model
downloaded from
http://www.cwru.edu/med/CBRTC/LRdOnline/development.htm

Modified by Keiji Imoto to make the program object-oriented
15-Dec-2003

#include "LRc.h"

CMyocyte::CMyocyte()
{
    // constant values
    R = 8314;    // Universal Gas Constant (J/kmol*K)
    frdy = 96485; // Faraday's Constant (C/mol)
    temp = 310;  // Temperature (K)
    pi = 3.141592;  // Pi

    // Ion Valences
    zna = 1;    // Na valence
    zk = 1;     // K valence
    zca = 2;    // Ca valence

    // Cell Geometry
    l = 0.01;   // Length of the cell (cm)
    a = 0.0011; // Radius of the cell (cm)
    vcell = 1000*pi*a*a*l;  // 3.801e-5 uL
    ageo = 2*pi*a*a+2*pi*a*l; // 7.671e-5 cm^2
    acap = ageo*2;  // 1.534e-4 cm^2
    vmyo = vcell*0.68;
    vmito = vcell*0.26;
    vsr = vcell*0.06;
    vnsr = vcell*0.0552;
    vjsr = vcell*0.0048;
    vcleft = vcell*0.12/0.88;

//===--------------------------------------------------
// time
    dt = udt;
    utsc = 50;

// output control
printdata = 0;
printval = 0;

// Ion Concentrations
// -- bulk medium
nabm = 140:  // Initial Bulk Medium Na (mM)
kbm = 4.5:   // Initial Bulk Medium K (mM)
  // kbm = 2.0:   // Initial Bulk Medium K (mM)
cabm = 1.8:  // Initial Bulk Medium Ca (mM)
taudiff = 1000:  // Diffusion Constant for Ion Movement from Bulk Medium to Cleft Space
// -- external
nao = 140:  // Initial Extracellular Na (mM)

// Change ko for external K concentration
// The normal default value is 4.5.
// ko = 4.5:   // Initial Extracellular K (mM)
ko = 4.5:
cao = 1.8:   // Initial Extracellular Ca (mM)
dnao = 0:
dko = 0:
dcao = 0:
// -- internal
nai = 9:    // Initial Intracellular Na (mM)
ki = 141.2: // Initial Intracellular K (mM)
cai = 0.00006:    // Initial Intracellular Ca (mM)
cmdn = 0.00257849: // Calmodulin Buffered Ca Concentration (mM)
trpn = 0.0143923: // Troponin Buffered Ca Concentration (mM)
nsr = 1.838:   // NSR Ca Concentration (mM)
jsr = 1.838:   // JSR Ca Concentration (mM)
csqn = 6.97978:  // Calsequestrin Buffered Ca Concentration (mM)

naiont = 0:;  // Total Na Ion Flow (uA/uF)
dnai = 0:    // Change in Intracellular Na Concentration (mM)
kiont = 0:;  // Total K Ion Flow (uA/uF)
dki = 0:    // Change in Intracellular K Concentration (mM)

// stimulation
st = 0:    // Stimulus
tstim = 10:  // Time to begin stimulus
stintime = 10:  // Initial Condition for Stimulus
bcl = 0:    // BCL:

// Voltage
v = -90:  // Initial Voltage (mv)
it = 0:   // total current
dvdt = 0:  // force to calculate currents at the beginning
dvdtmax = dvdt:
triggerCICR = false: // flag for Ca release

// Fast Sodium Current (time dependant)
gna = 16:
m = 0.0008: // Na activation
h = 0.993771: // Na inactivation
j = 0.995727: // Na inactivation

// Current through L-type Ca Channel
kmca = 0.0006: // Half-saturation concentration of Ca channel (mM)
pca = 0.00054: // Permiability of membrane to Ca (cm/s)
gacai = 1: // Activity coefficient of Ca
gacao = 0.341: // Activity coefficient of Ca
pna = 0.000000675: // Permiability of membrane to Na (cm/s)
ganai = 0.75: // Activity coefficient of Na
ganao = 0.75: // Activity coefficient of Na
pk = 0.000000193: // Permiability of membrane to K (cm/s)
gaki = 0.75: // Activity coefficient of K
gako = 0.75: // Activity coefficient of K

prnak = 0.01833: // Na/K Permiability Ratio
gkna = 0.12848: // Maximum conductance (mS/uF)
nkna = 2.8: // Hill coefficient for Na dependance
kdka = 66: // Dissociation constant for Na dependance (mM)
natp = 0.24: // K dependence of ATP-sensitive K current
nicholsarea = 0.00005: // Nichol’s ares (cm^2)
atpi = 3: // Intracellular ATP concentration (mM)
hatp = 2: // Hill coefficient
katp = 0.250: // Half-maximal saturation point of ATP-sensitive K current (mM)

//*** PUMPS ***
c1 = .00025: // Scaling factor for inaca (uA/uF)
c2 = 0.0001: // Half-saturation concentration of NaCa exhanger (mM)
gammas = .15: // Position of energy barrier controlling voltage dependance of inaca

imbarnak = 2.25: // Max. current through Na-K pump (uA/uF)
kmannai = 10: // Half-saturation concentration of NaK pump (mM)
kmno = 1.5: // Half-saturation concentration of NaK pump (mM)
pnsca = 0.000000175: // Permiability of channel to Na and K (cm/s)
kmsnc = 0.0012: // Half-saturation concentration of NSCa channel (mM)
ibarpca = 1.15: // Max. Ca current through sarcolemmal Ca pump (uA/uF)
kmipca = 0.0005: // Half-saturation concentration of sarcolemmal Ca pump (mM)
ggap = 0.1:

// Initial Gate Conditions
d = 3.210618e-06:
f = 0.999837;
xs1 = 0.00445683;
xs2 = 0.00445683;
xr = 0.000124042;
b = 0.000970231;
g = 0.994305;
zdv = 0.0120892;
ydv = 0.999978;

// *** SR ***
// NSR Ca Ion Concentration Changes
kmup = 0.00092; // Half-saturation concentration of iup (mM)
iupbar = 0.00875; // Max. current through iup channel (mM/ms)
nsrbar = 15; // Max. [Ca] in NSR (mM)

// JSR Ca Ion Concentration Changes
tauon = 2; // Time constant of activation of Ca release from JSR (ms)
tauoff = 2; // Time constant of deactivation of Ca release from JSR (ms)
csqnth = 8.75; // Threshold for release of Ca from CSQN due to JSR overload (mM)
gmaxrel = 150; // Max. rate constant of Ca release from JSR due to overload (mM/ms^-1)
csqnbar = 10; // Max. [Ca] buffered in CSQN (mM)
kmcsqn = 0.8; // Equilibrium constant of buffering for CSQN (mM)

// Translocation of Ca Ions from NSR to JSR
itr = 0; // Translocation current of Ca ions from NSR to JSR (mM/ms)
tautr = 180; // Time constant of Ca transfer from NSR to JSR (ms)

// Myoplasmic Ca Ion Concentration Changes
cmdnbar = 0.050; // Max. [Ca] buffered in CMDN (mM)
trpnbar = 0.070; // Max. [Ca] buffered in TRPN (mM)
kmcmdn = 0.00238; // Equilibrium constant of buffering for CMDN (mM)
kmtrpn = 0.0005; // Equilibrium constant of buffering for TRPN (mM)

// Ca release
grelbarjsrol = 0;
tjsrol = 25;
tcicr = 25;
dcaiont = 0;
}

void CMyocyte::setStepTime(double ut)
{
    udt = ut;
    dt = udt;
}

CMyocyte* CMyocyte::getPrevCell(int ci)
{
if (ci==0) return NULL;
return &pCellArray[ci-1];
}

CMyocyte* CMyocyte::getNextCell(int ci)
{
    if (ci>=nCells-1) return NULL;
    return &pCellArray[ci+1];
}

bool CMyocyte::checkChanges(double t)
{
    // nxstep = (fabs(dvdt)<0.25 && v<0) ? 500 : 50;
    nxstep = (fabs(dvdt)<0.25 && v<0) ? 50 : 25;

    if (utsc>=nxstep || dvdt>5 || irelcicr>0.01 || fabs(t-tstim)<=udt ||
        (stimtime>=0 && stimtime<0.5)){
        return true;
    }
    return false;
}

void CMyocyte::calculateChanges(double t)
{
    ena = ((R*temp)/frdy)*log(nao/nai);
    ekr = ((R*temp)/frdy)*log(ko/ki);

#if MARKOV_NA == 1
    ina = wtNaChannel.compCurrent(t, dt, v, ena);
    ina += mutantNaChannel.compCurrent(t, dt, v, ena);
#else
    comp_ina ();
#endif

    comp_ical ();
    comp_icat ();

#if MARKOV_HERG == 1
    ikr = HERGChannel.compCurrent(t,dt,v,ekr,ko);
#else
    comp_ikr ();
#endif

    comp_iks ();
    comp_iki ();
    comp_ikp ();
    ikna = 0;  //comp_ikna ();
    ikatp = 0;  //comp_ikatp ();
ito = 0;  //comp_ito ();
comp_inaca ();
comp_inak ();
insna = 0; insk = 0;  //comp_insca ();
comp_ipca ();
comp_icab ();
comp_inab ();
comp_gap ();
comp_it (t);
conc_nai ();
conc_ki ();
conc_nsr ();
conc_jsr ();
calc_itr ();
calc_cai ();
  //conc_cleft ();  // Cleft Space disabled, if you want to use cleft space, make sure the initial
  //conditions of ion concentrations in the bulk medium are the same as the extracellular concentraions
utsc = 0;
dt = 0;  // dt : dt used for calculating current changes
}

bool CMyocyte::advanceStep(double t) {
  printval = (dvdt>3 || irelcicr>.01) ? 50 : 750;
  printFlag = false;
  if(printdata>=printval) {
    printFlag = true;
    printdata = 0;
  }
  printdata++;

  if(csqn>=csqnth && tjsrol>50) {
    grelbarjsrol = 4;
    tjsrol = 0;
    printf("Spontaneous Release occured at time %lf\n", t);
  }

  vnew = v-it*udt;
  dvdtnew = (vnew-v)/udt;
  v = vnew;
  dvdt = dvdtnew;

  v -= it*udt;  // get new voltage
  dvdt = -it;

  //### added 10-Jan-2004
  if(dvdt >= dvdtmax)dvdtmax = dvdt;
if(dvdt<dvdtmax && dvdtmax > 100) triggerCICR = true;

//###

dt += udt;

caiontold = caiont;
dcaiont = dcaiontnew;

return printFlag;
}

//-------------
// Total sum of currents is calculated here.
// if the time is between stimtime = 0 and stimtime = 0.5,
// a stimulus is applied

void CMyocyte::comp_it (double t)
{
  naiont = ina+inab+ilcana+insna+3*inak+3*inaca;
kiont = ikr+iks+iki+ikp+ilcak+insk-2*inak+ito+ikna+ikatp+igap;
caiont = ilca+icab+ipca-2*inaca+icat;

it = naiont+kiont+caiont;

if(st == 0) return;

if (t>=tstim && t<(tstim+dt)){
  it += st;
}

if(stimtime>=0 && stimtime<0.5 && t > bcl) it += st;

stimtime += dt;
}

//******************************************
// Functions that describe the currents begin here
//
// gap junction:
void CMyocyte::comp_gap ()
{
  CMyocyte* pCell;
pCell = getPrevCell(cellIndex);
igap = (pCell==NULL) ? 0 : ggap*(v - pCell->v);

pCell = getNextCell(cellIndex);
igap += (pCell==NULL) ? 0 : ggap*(v - pCell->v);
}

void CMyocyte::comp_ina ()
{
  ena = ((R*temp)/frdy)*log(nao/nai);
am = 0.32*(v+47.13)/(1-exp(-0.1*(v+47.13)));
bm = 0.08*exp(-v/11);

  if (v < -40) {
    ah = 0.135*exp((80+v)/-6.8);
bh = 3.56*exp(0.079*v)+310000*exp(0.35*v);
    aj = (-127140*exp(0.2444*v)-0.00003474*exp(-0.04391*v))*((v+37.78)/(1+exp(-0.311*(v+79.23))));
bj = (0.1212*exp(-0.01052*v))/(1+exp(-0.1378*(v+40.14)));
  }else{
    ah = 0;
bh = 1/(0.13*(1+exp((v+10.66)/-11.1)));
    aj = 0;
    bj = (0.3*exp(-0.000002535*v))/(1+exp(-0.1*(v+32)));
  }

  h = ah/(ah+bh)-((ah/(ah+bh))-h)*exp(-dt/(1/(ah+bh)));
  j = aj/(aj+bj)-((aj/(aj+bj))-j)*exp(-dt/(1/(aj+bj)));
  m = am/(am+bm)-((am/(am+bm))-m)*exp(-dt/(1/(am+bm)));

  ina = gna*m*m*h*j*(v-ena);
}

void CMyocyte::comp_ical ()
{
  dss = 1/(1+exp(-(v+10)/6.24));
taud = dss*(1-exp(-(v+10)/6.24))/(0.035*(v+10));

  fss = (1/(1+exp((v+32)/8)))+(0.6/(1+exp((50-v)/20)));
tauf = 1/(0.0197*exp(-pow(0.0337*(v+10),2))+0.02);

  d = dss-(dss-d)*exp(-dt/taud);
f = fss-(fss-f)*exp(-dt/tauf);

  ibarca = pca*zca*zca*((v*frdy*frdy)/(R*temp))*((gacai*cai*exp((zca*v*frdy)/(R*temp))-gacao*cao)/(exp((zca*v*frdy)/(R*temp))-1));
ibarna = pna*zna*zna*((v*frdy*frdy)/(R*temp))*((ganai*nai*exp((zna*v*frdy)/(R*temp)) - ganao*nao)/(exp((zna*v*frdy)/(R*temp)) - 1));

ibark = pk*zk*zk*((v*frdy*frdy)/(R*temp))*((gaki*ki*exp((zk*v*frdy)/(R*temp)) - gako*ko)/(exp((zk*v*frdy)/(R*temp)) - 1));

fca = 1/(1+cai/kmca);

ilca = d*f*fca*ibarca;
ilcana = d*f*fca*ibarna;
ilcak = d*f*fca*ibark;
ilcatot = ilca+ilcana+ilcak;

}  
void CMyocyte::comp_icat ()  
{  
  bss = 1/(1+exp(-(v+14)/10.8));
taub = 3.7+6.1/(1+exp((v+25)/4.5));
gss = 1/(1+exp((v+60)/5.6));
  if (v<=0) {
    taug = -0.875*v+12;
  }else{
    taug = 12;
  }

  b = bss-(bss-b)*exp(-dt/taub);
g = gss-(gss-g)*exp(-dt/taug);

  gcat = 0.05;
eca = (R*temp/(2*frdy))*log(cao/cai);
icat = gcat*b*b*g*(v-eca);
}

void CMyocyte::comp_ikr ()  
{  
gkr = 0.02614*sqrt(ko/5.4);
ekr = ((R*temp)/frdy)*log(ko/ki);

  xrss = 1/(1+exp(-(v+21.5)/7.5));
tauxr = 1/(0.00138*(v+14.2)/(1-exp(-0.123*(v+14.2)))+0.00061*(v+38.9)/(exp(0.145*(v+38.9))-1));
  xr = xrss-(xrss-xr)*exp(-dt/tauxr);

  r = 1/(1+exp((v+9)/22.4));
ikr = gkr*xr*r*(v-ekr);
}

void CMyocyte::comp_iks ()
{
gks = 0.202*(1+0.6/(1+pow((0.000038/cai),1.4)));
eks = ((R*temp)/frdy)*log((ko+prnak*nao)/(ki+prnak*nai));

xs1ss = 1/(1+exp(-(v-1.5)/16.7));
xs2ss = xs1ss;

tauxs1 = 1/(0.0000719*(v+30)/(1-exp(-0.148*(v+30)))+0.000131*(v+30)/((exp(0.0687*(v+30))-1));
tauxs2 = 4*tauxs1;

xs1 = xs1ss-(xs1ss-xs1)*exp(-dt/tauxs1);
xs2 = xs2ss-(xs2ss-xs2)*exp(-dt/tauxs2);

iks = gks*xs1*xs2*(v-eks);
}

void CMyocyte::comp_iki ()
{
gki = 0.75*(sqrt(ko/5.4));
eki = ((R*temp)/frdy)*log(ko/ki);

aki = 1.02/(1+exp(0.2385*(v-eki-59.215)));
bki = (0.49124*exp(0.08032*(v-eki+5.476)) + exp(0.06175*(v-eki-594.31)))/(1+exp(-0.5143*(v-eki+4.753)));

kin = aki/(aki+bki);
iki = gki*kin*(v-eki);
}

void CMyocyte::comp_ikp ()
{
gkp = 0.00552;
epk = eki;

kp = 1/(1+exp((7.488-v)/5.98));

ikp = gkp*kp*(v-ekp);
void CMyocyte::comp_ikna ()
{
    ekna = ((R*temp)/frdy)*log(ko/ki);
    pona = 0.85/(1+pow((kdkna/nai),2.8));
    pov = 0.8-0.65/(1+exp((v+125)/15));
    ikna = gkna*pona*pov*(v-ekna);
}

void CMyocyte::comp_ikatp ()
{
    // Note: If you wish to use this current in your simulations, there are additional
    // changes which must be made to the code as detailed in Cardiovasc Res 1997:35:256-272

    ekatp = ((R*temp)/frdy)*log(ko/ki);
    gkatp = 0.000195/nicholsarea;
    patp = 1/(1+pow((atpi/katp),hatp));
    gkbaratp = gkatp*patp*(pow((ko/4),natp));

    ikatp = gkbaratp*(v-ekatp);
}

void CMyocyte::comp_ito ()
{
    gitodv = 1.1;
    ekdv = ((R*temp)/frdy)*log((ko)/(ki));
    rvdv = exp(v/100);

    azdv = (10*exp((v-40)/25))/(1+exp((v-40)/25));
    bzdv = (10*exp(-(v+90)/25))/(1+exp(-(v+90)/25));
    tauzdv = 1/(azdv+bzdv);
    zssdv = azdv/(azdv+bzdv);
    zdv = zssdv-(zssdv-zdv)*exp(-dt/tauzdv);

    aydv = 0.015/(1+exp((v+60)/5));
    bydv = (0.1*exp((v+25)/5))/(1+exp((v+25)/5));
    tauydv = 1/(aydv+bydv);
    yssdv = aydv/(aydv+bydv);
    ydv = yssdv-(yssdv- ydv)*exp(-dt/taudydv);

    ito = gitodv*zdvrzdvydv*rvdv*(v-ekdv);
}
```cpp
void CMyocyte::comp_inaca ()
{
    inaca =
    c1*exp((gammas-1)*v*frdy/(R*temp)) * ((exp(v*frdy/(R*temp)) * nai*nai*nai*cao-nao*nao*nao*cai)/(1+
    c2*exp((gammas-1)*v*frdy/(R*temp)) * (exp(v*frdy/(R*temp)) * nai*nai*nai*cao+nao*nao*nao*cai)));}

void CMyocyte::comp_inak ()
{
    sigma = (exp(nao/67.3)-1)/7;
    fnak = 1/(1+0.1245*exp((-0.1*v*frdy)/(R*temp))+0.0365*sigma*exp((-v*frdy)/(R*temp)));
    inak = ibarnak*fnak*(1/(1+pow(kmnai/nai,2)))*(ko/(ko+kmko));
}

void CMyocyte::comp_insca ()
{
    ibarnsna =
    pnsca*zna*zna*((v*frdy*frdy)/(R*temp))*((ganai*nai*exp((zna*v*frdy)/(R*temp)) - ganao*nao)/(exp(
    (zna*v*frdy)/(R*temp)) -1));
    ibarnsk =
    pnsca*zk*zk*((v*frdy*frdy)/(R*temp))*((gaki*ki*exp((zk*v*frdy)/(R*temp)) - gako*kko)/(exp((zk*v*frdy)/(R*temp)) -1));
    insna = ibarnsna/(1+pow(kmnsca/cai,3));
    insk = ibarnsk/(1+pow(kmnsca/cai,3));
}

void CMyocyte::comp_ipca ()
{
    ipca = (ibarpca*cai)/(km pca+cai);
}

void CMyocyte::comp_icab ()
{
    gcab = 0.003016;
    ecan = ((R*temp)/(2*frdy))*log(cao/cai);
    icab = gcab*(v-ecan);
}

void CMyocyte::comp_inab ()
{
    gnab = 0.004;
    enan = ena;
    inab = gnab*(v-enan);
}

// Functions that calculate intracellular ion concentrations begins here
```
void CMyocyte::conc_nai ()
{
    dnai = -dt*(naiont*acap)/(vmyo*zna*frdy);
    nai = dnai + nai;
}

void CMyocyte::conc_ki ()
{
    if(stimtime>=0 && stimtime<0.5){
        dki = -dt*((kiont+st)*acap)/(vmyo*zk*frdy);
    }
    else{
        dki = -dt*(kiont*acap)/(vmyo*zk*frdy);
    }
    ki = dki + ki;
}

// NSR Ca Ion Concentration Changes
// kmup = 0.00092;    // Half-saturation concentration of iup (mM)
// iupbar = 0.00875;  // Max. current through iup channel (mM/ms)
// nsrbar = 15;       // Max. [Ca] in NSR (mM)
// kleak;  Rate constant of Ca leakage from NSR (ms^-1)
// nsr     NSR Ca Concentration (mM)
void CMyocyte::conc_nsr ()
{
    kleak = iupbar/nsrbar;
    ileak = kleak*nsr;
    iup = iupbar*cai/(cai+kmup);
    dnsr = dt*(iup-ileak-itr*vjsr/vnsr);
    nsr = nsr+dnsr;
}

// void CMyocyte::conc_jsr ()
{
    dcaiontnew = (caiont-caiontold)/dt;

    if(v>-35 && dcaiontnew>dcaiont && triggerCICR){
        dvdtmax = 0;  //### added 10-Jan-2004
        triggerCICR = false;
        tcicr = 0;
    }
    // Release due to CICR
\[ RyRopen = \frac{1}{1 + \exp((-tcicr+4)/0.5)}; \]
\[ RyRclose = \frac{1}{1 - 1/(1 + \exp((-tcicr+4)/0.5))}; \]
\[ grel = \frac{gmaxrel}{1 + \exp((i\text{loa} + i\text{cab} + i\text{pca} - 2*i\text{naca} + i\text{cat}) + 5)/0.9}); \]
\[ i\text{relcicr} = grel \times RyRopen \times RyRclose \times (\text{jsr} - \text{cai}); \]
\[ tcicr = tcicr + dt; \]

// Release due to overload
\[ grel\text{jsrol} = grel\text{barjsrol} \times (1 - \exp(-t\text{jsrol}/\tau\text{auon})) \times \exp(-t\text{jsrol}/\tau\text{off}); \]
\[ irel\text{jsrol} = grel\text{jsrol} \times (\text{jsr} - \text{cai}); \]
\[ t\text{jsrol} = t\text{jsrol} + dt; \]

\[ c\text{sqn} = c\text{sqnbar} \times (\text{jsr}/(\text{jsr} + \text{kmcsqn})); \]
\[ d\text{jsr} = dt \times (\text{itr} - irel\text{cicr} - irel\text{jsrol}); \]
\[ b\text{jsr} = c\text{sqnbar} - c\text{sqn} - d\text{jsr} - \text{jsr} + \text{kmcsqn}; \]
\[ c\text{jsr} = \text{kmcsqn} \times (c\text{sqn} + d\text{jsr} + \text{jsr}); \]
\[ \text{jsr} = (\sqrt{(b\text{jsr} \times b\text{jsr} + 4 \times c\text{jsr})} - b\text{jsr})/2; \]

\[ \text{itr} = (\text{nsr} - \text{jsr})/\tau\text{utr}; \]

\[ \text{dcai} = -dt * ((\text{cai}\text{ont} \times \text{acap})/(\text{vmoyo} \times \text{zca} \times \text{frdy})) + ((\text{iup} - \text{ileak}) \times \text{vnsr}/\text{vmoyo}) - (irel\text{cicr} \times \text{vjsr}/\text{vmoyo}) - (irel\text{jsrol} \times \text{vjsr}/\text{vmoyo}); \]
\[ \text{trpn} = \text{trpnbar} \times (\text{cai}/(\text{cai} + \text{kmtrpn})); \]
\[ \text{cmdn} = \text{cmdnbar} \times (\text{cai}/(\text{cai} + \text{kmcmdn})); \]
\[ \text{catotal} = \text{trpn} + \text{cmdn} + \text{dcai} + \text{cai}; \]
\[ \text{bmyo} = \text{cmdnbar} + \text{trpnbar} - \text{catotal} + \text{kmtrpn} + \text{kmcmdn}; \]
\[ \text{cmyo} = (\text{kmcmdn} \times \text{kmtrpn}) - (\text{catotal} \times (\text{kmtrpn} + \text{kmcmdn})) + (\text{trpnbar} \times \text{kmcmdn}) + (\text{cmdnbar} \times \text{kmtrpn}); \]
\[ \text{dmyo} = -\text{kmtrpn} \times \text{kmcmdn} \times \text{catotal}; \]
\[ \text{gpig} = \sqrt{\text{bmyo} \times \text{bmyo} - 3 \times \text{cmyo}}; \]
\[ \text{cai} = (2 \times \text{gpig}/3) \times \cos(\text{acos}((9 \times \text{bmyo} \times \text{cmyo} - 2 \times \text{bmyo} \times \text{bmyo} - 27 \times \text{dmyo})/(2 \times \text{pow}((\text{bmyo} \times \text{bmyo} - 3 \times \text{cmyo}), 1.5))))/3 - (\text{bmyo}/3); \]

\[ \text{dnao} = dt \times ((\text{na}\text{mb} - \text{na}\text{o})/\tau\text{diff} + \text{na}\text{iont} \times \text{acap})/(\text{vclleft} \times \text{frdy})); \]
\[ \text{nao} = \text{dnao} + \text{nao}; \]
\[ \text{dko} = dt \times ((\text{kbo} - \text{ko})/\tau\text{diff} + \text{kiont} \times \text{acap})/(\text{vclleft} \times \text{frdy})); \]
\[ \text{ko} = \text{dko} + \text{ko}; \]
\[ \text{dcao} = dt \times ((\text{cabm} - \text{cao})/\tau\text{diff} + \text{caiont} \times \text{acap})/(\text{vclleft} \times \text{frdy} \times 2)); \]
\[ \text{cao} = \text{dcao} + \text{cao}; \]
Source Files (MarkovHERGChannel.cpp)

//
//      C3  y[0]
//      C2  y[1]
//      C1  y[2]
//      O   y[3]
//      I   y[4]

#include "LRc.h"

CMarkovHERGChannel::CMarkovHERGChannel() {
    nStates = NUM_OF_HERG STATES;
    gherg = 0;
    for (ix=0;ix<nStates;ix++) pHERG[ix] = 0;
    pHERG[0] = 1;

    for (ix=0;ix<nStates;ix++) {
        y[ix]=0;
        yn[ix]=0;
        k[ix]=0;
        dny[ix]=0;
        dny1[ix]=0;
    }
}

CMarkovHERGChannel::~CMarkovHERGChannel() {
}

void CMarkovHERGChannel::getdy(double dt, double* dy) {
    k[0] = -a*y[0] + b*y[1];
    k[1] = -(b + ain)*y[1] + a*y[0] + bin*y[2];

    for (int ix=0;ix<nStates;ix++) dy[ix]= k[ix]*dt;
}

void CMarkovHERGChannel::getRateConstants(double v) {
    aa  = 65.5e-3 * exp(0.05547153*(v-36)); // C1-->O, C1-->I
    ain = 2.172; // C2-->C1
\[ a = 55.5 \times 10^{-3} \times \exp(0.05547153 \times (v - 12)) \quad \text{// C3\rightarrow C2} \]
\[ b = 2.357 \times 10^{-3} \times \exp(-0.036588 \times v) \quad \text{// C2\rightarrow C3} \]
\[ \text{bin} = 1.077 \quad \text{// C1\rightarrow C2} \]
\[ \text{bb} = 2.9357 \times 10^{-3} \times \exp(-0.02352 \times (v+25)) \times 4.5 / Kout \quad \text{// I\rightarrow C1} \]
\[ ai = 0.439 \times \exp(-0.02352 \times (v+25)) \times 4.5 / Kout \quad \text{// I\rightarrow C1d} \]
\[ bi = 0.656 \times \exp(0.000942 \times v) \times \text{pow}(4.5 / Kout, 0.3) \quad \text{// 3*O\rightarrow C3} \]
\[ \mu = (ai \times bb \times aa) / (aa \times bi) \quad \text{// I\rightarrow C1d} \]

```cpp
double CMarkovHERGChannel::compCurrent(double t, double dt, double v, double ek, double Kout) // current function
{
    Kout = Kout;
    getRateConstants(v);
    i4=0;
    while(1)
    {
        if(i4==0)
        {
            for(ix=0;ix<nStates;ix++)
                y[ix] = pHERG[ix]; // mempcpy(y,pHERG,sizeof(double)*nStates);
            getdy(dt, dny1);
            for(ix=0;ix<nStates;ix++)
                yn[ix] = y[ix] + dny1[ix];
        }
        else
        {
            for(ix=0;ix<nStates;ix++)
                y[ix] = yn[ix]; // mempcpy(y,yn,sizeof(double)*nStates);
            getdy(dt, dny);
            for(ix=0;ix<nStates;ix++)
            {
                dny[ix] = (dny[ix] + dny1[ix])/2;
                yn[ix] = pHERG[ix] + dny[ix];
                dny1[ix] = dny[ix];
            }
        }
    }
    sum = 0;
    for(ix=0;ix<nStates-1;ix++)
        sum += yn[ix];
    yn[nStates-1] = 1-sum; // last element
    done = true;
    for(ix=0;ix<nStates;ix++)
    {
        if(fabs(yn[ix] - y[ix])>1e-5)
        {
            done = false; // not done yet
            break;
        }
    }
    if(done) break; // really done
    i4++;
    if (i4>=40) {
        printf("? error in Markov Na at %g: i4 = %d, item=%d, yn", t, i4, ix);
    }
}
```
exit(0);
}
} // end of while

for (ix=0;ix<nStates;ix++) pHERG[ix] = yn[ix];
// memcpy(pHERG,yn,sizeof(double)*nStates);

gherg = 0.0135*pow(Kout, 0.59);

    return gherg * pHERG[OPEN_STATE] * (v-ek);
}
// Na channel Markov process model
//
// IC3 - IC2 - IF - IM1 - IM2
//    |   |   /  |
// C3 - C2 - C1 - 0
//
// xd[0]  C3
// xd[1]  C2
// xd[2]  C1
// xd[3]  0
// xd[4]  IC3
// xd[5]  IC2
// xd[6]  IF
// xd[7]  IM1
// xd[8]  IM2
//
// Integration methods
// modified from Colleen Clancy's method
// http://www.cwru.edu/med/CBRTC/LRdOnline/markovina.htm
//

#include "LRc.h"

CMarkovNaChannel::CMarkovNaChannel()
{
    nStates = (bBurstMode)? NUM_OF_NA_STAT + NUM_OF_NA_BURST_STATES : NUM_OF_NA_STAT;
    gna = 16;
    for(ix=0;ix<nStates;ix++)pNa[ix] = 0;
    pNa[0] = 1;
}

CMarkovNaChannel::~CMarkovNaChannel()
{
}

void CMarkovNaChannel::getdy(double dt, double* dy)
{
    if(bBurstMode)
    {
        k[0] = -(a11 + b3 + a6)*y[0] + b11*y[1] + a3*y[4] + b6*y[9];
    }
}
\[ k[4] = -(a11 + a3) \cdot y[4] + b11 \cdot y[5] + b3 \cdot y[0]; \]
\[ k[7] = -(b4 + a5) \cdot y[7] + a4 \cdot y[6] + b5 \cdot y[8]; \]
\[ k[8] = -b5 \cdot y[8] + a5 \cdot y[7]; \]
\[ k[9] = -(a11 + b6) \cdot y[9] + b11 \cdot y[10] + a6 \cdot y[0]; \]
\[ k[12] = -(b13 + b6) \cdot y[12] + a13 \cdot y[11] + a6 \cdot y[3]; \]
\[ // \]
\[ ]\]
\[ else \{
\[ k[0] = -(a11 + b3) \cdot y[0] + b11 \cdot y[1] + a3 \cdot y[4]; \]
\[ k[1] = -(b11 + b3 + a12) \cdot y[1] + a11 \cdot y[0] + b12 \cdot y[2] + a3 \cdot y[5]; \]
\[ k[3] = -(b13 + a2) \cdot y[3] + a13 \cdot y[2] + b2 \cdot y[6]; \]
\[ k[4] = -(a11 + a3) \cdot y[4] + b11 \cdot y[5] + b3 \cdot y[0]; \]
\[ k[7] = -(b4 + a5) \cdot y[7] + a4 \cdot y[6] + b5 \cdot y[8]; \]
\[ k[8] = -b5 \cdot y[8] + a5 \cdot y[7]; \]
\[ ]\]

for (int ix=0;ix<nStates;ix++) dy[ix] = k[ix]*dt;

void CMarkovNaChannel::getRateConstants(double v) {
    a11 = 3.802/(0.1027*exp(-v/17.0) + 0.20*exp(-v/150));
    a12 = 3.802/(0.1027*exp(-v/15.0) + 0.23*exp(-v/150));
    a13 = 3.802/(0.1027*exp(-v/12.0) + 0.25*exp(-v/150));
    b11 = 0.1917*exp(-v/20.3);
    b12 = 0.20*exp(-v-5)/20.3;
    b13 = 0.22*exp(-v-10)/20.3;
    a2 = 3*(7933e-7)*exp(-v/7.7); // (3.7933e-7)*exp(-v/7.7);
    b3 = 0.0084 + 0.000002*v;
    a3 = (3.7933e-7)*exp(-v/7.7); // (3.7933e-7)*exp(-v/7.7);
    b4 = a3;
    a4 = a2/100;
    b5 = 3/50://a3/50;
    if (bBurstMode) {
        a6 = 1000e-4;
    }
}
double CMarkovNaChannel::compCurrent(double t, double dt, double v, double ena) // current function
{
    getRateConstants(v);
    double b6 = 3e-4;

    i4=0;
    while(1){
        if(i4==0){
            for(ix=0;ix<nStates;ix++) y[ix] = pNa[ix];
            getdy(dt, dny1);
            for(ix=0;ix<nStates;ix++) yn[ix] = y[ix] + dny1[ix];
        } else{
            for(ix=0;ix<nStates;ix++) y[ix] = yn[ix];
            getdy(dt, dny);
            for(ix=0;ix<nStates;ix++){
                dny[ix] = (dny[ix] + dny1[ix])/2;
                yn[ix] = pNa[ix] + dny[ix];
                dny1[ix] = dny[ix];
            }
        }

        sum = 0;
        for(ix=0;ix<nStates-1;ix++) sum += yn[ix];
        yn[nStates-1] = 1-sum; // last element

        done = true;
        for(ix=0;ix<nStates;ix++){ // check difference
            if(fabs(yn[ix] - y[ix])>1e-5){
                done = false; // not done yet
                break;
            }
        }
        if(done) break; // really done

        i4++;
        if (i4>=40) {
            printf("? error in Markov Na at %g: i4 = %d, item=%d, Yn", t, i4, ix);
            exit(0);
        }
    } // end of while

    for(ix=0;ix<nStates;ix++) pNa[ix] = y[ix];

    if(bBurstMode){
        return gna * (pNa[OPEN_STATE] + pNa[OPEN_STATE_B]) * (v-ena);
    } else{

return gna * pNa[OPEN_STATE] * (v-ena);
Supplemental data 2.

Case 2 with M756V/KCNH2

A 63-year-old man was admitted because of syncope. He had no past history of syncope or family history of sudden cardiac death or LQTS. He had been taking pirmenol (300 mg per day) for several years for the treatment of paroximal supraventricular tachycardia. An ECG taken on admission showed repetitive TdP preceded by the prolongation of QTc interval (632 ms). Pirmenol intake was immediately stopped, and his TdP was successfully treated by an infusion of lidocaine and magnesium sulfate. Afterwards, he underwent catheter ablation for the accessory pathway (under the diagnosis of concealed Wolff-Parkinson-White [WPW] syndrome) and his QT interval shortened to 453 ms.

Case 4 with H492Y/KCNH2

A 52-year-old woman experienced syncope while taking disopyramide (300 mg/day) for the treatment of frequent premature ventricular contractions. She had no family history of premature sudden death or long QT syndrome (LQTS). Her ECG on admission showed a prolonged QTc interval (585 ms), followed by repetitive TdP. Serum potassium was low (3.2 mEq/ml). The combined infusion of magnesium sulfate and lidocaine failed to terminate TdP. However, TdP did not recur after she stopped taking disopyramide and started taking an oral potassium preparation. At that time, her QTc interval shortened to 447 ms. Treadmill exercise test, performed in the absence of disopyramide, induced neither the prolongation of QT interval nor ventricular arrhythmia.

Case 14 with R243H/KCNQ1

A 52-year-old woman was admitted to hospital because of repetitive syncope. She had been taking aprindine (60mg/day), mexiletine (400mg/day) and propranolol (30mg/day) for the treatment of ventricular arrhythmia. She experienced frequent episodes of TdP with prolonged QT interval (857ms). However, the QT interval shortened to 469 ms after discontinuing aprindine. After a follow-up of 3 years there have been no further TdP attacks; only aprindine was permanently discontinued.

Case 15 with S706F/KCNH2

A 21-year-old woman was admitted for emergency care because of palpitations and dyspnea. She had no history of syncope. A previous ECG taken at age 15 years had shown normal QTc
interval (430 ms). There was no family history of sudden cardiac death or LQTS. Her symptoms appeared about 20 minutes after she took 10 combination tablets of amphetamine and methamphetamine. Her ECG showed repeated TdP with the prolongation of QTc interval (600 ms). She was treated firstly with an infusion of lidocaine (100 mg), followed by an infusion of magnesium sulfate (20mEq/day). However, TdP still occurred at a low heart rate (55 bpm), and temporary ventricular pacing was carried out to ensure a minimum heart rate of 70 bpm. The latter procedure suppressed her TdP. After washout of amphetamines, her QT interval shortened to 450 ms.

**Case 16 with L1825P/SCN5A**

This 70-year-old woman had TdP and the prolongation of QT interval to 731 ms after taking cisapride and pirmenol. This case had been reported in a previous manuscript.1

**Case 17 with D342V/KCNH2**

A 70-year-old woman was admitted to hospital because of syncope during lunch. She had been taking erythromycin (1200mg/day) for the treatment of acute bronchitis and bronchoectasis for 2 weeks, and had no history of syncope. Her sister had died suddenly at the age of 24. ECG recorded in the ambulance documented repetitive TdP and a prolonged QTc interval of 776 ms. Serum electrolytes were normal. TdP was terminated by an intravenous infusion of magnesium sulfate (80 mEq/day) with atrial pacing at a rate of 100 bpm. After stopping erythromycin, her QTc interval shortened to 440 ms.

**Case 18 with R231C/KCNQ1**

A 72-year-old woman was admitted to the hospital because of pre-syncope. TdP, with a prolonged QT interval of 754 ms, was apparent on her ECG. She had been taking probucol (250mg/day) for hypercholesterolemia. After discontinuing probucol, her QT interval did not normalize although it was shortened to 495 ms, and therefore the decision was taken to implant an implantable cardioverter defibrillator (ICD).

**Case 20 with A614V/KCNH2**

A 34-year-old woman experienced syncope, with unknown etiology, 3 days after starting hydroxyzine (3mg/day) for chronic urticaria; the QT interval was prolonged to 626 ms. There was no family history of LQTS or sudden cardiac death. She had syncope and the QT interval prolonged to 626 ms after 3 days. Three weeks after stopping hydroxyzine, the QT interval shortened to 449 ms. Detailed information on this case, including pharmacological characteristics
has been submitted in another paper.  

References


<table>
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<tr>
<th>Genes</th>
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<td>SCN5A</td>
<td>L619F</td>
<td>1/1</td>
<td>0</td>
<td>538</td>
<td>Wehrens XH, et al. Hum Mutat 2003; 21:552.</td>
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<tr>
<td>SCN5A</td>
<td>A1330P</td>
<td>1/1</td>
<td>0</td>
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