Relevance of the KCNH2 Protein Stoichiometry to Pathological Conditions Underlying QT Abnormality

C Wang[1], P Beyerlein[2], G Petznick[2], A Krause[2], C Nugent[1], W Dubitzky[1]

[1]University of Ulster, UK
[2]University of Applied Sciences Wildau, Germany

Abstract

Genetic defects in the KCNH2 gene are a primary cause of instable cardiac ventricular repolarization. The aim of this work has been to assess the functional implication of the stoichiometric properties of the mutated KCNH2 protein complex. We have developed both homotetrameric and heterotetrameric kinetic models based on the heterologous expression of wild-type KCNH2 and/or C-terminus mutants in HEK293 cells, and incorporated defined deterministic and stochastic tetramerizations into the Luo-Rudy dynamic model. In comparison with WT homomeric channels, homomeric mutated channels showed a propensity to develop a shorter action potential duration (APD). As a consequence of the interplay between trafficking deficiency and altered kinetics a random tetramerization of WT and mutant subunits resulted in a marked prolongation of APD. These results suggest that stoichiometry of the KCNH2 channel protein complex may be a substrate for variable genetic penetrance in long QT syndrome.

1. Introduction

Inherited long QT syndromes (LQTS) have been linked to genetic defects in several cardiac ion channels and one of them is the rapid delayed rectifier potassium channel (I_{kr}). The physical composition of I_{kr} consists of a tetramer of four identical KCNH2 (HERG) subunits, modulated by an auxiliary regulator encoded by the KCNE2 gene. Since the addition of hKCNE2 to the KCNH2 tetramer has shown less impact on the kinetics except a suppression of the current density in the co-expressed protein complex. The heterologous expression of KCNH2 in *Xenopus* oocytes or HEK cell lines has been widely used to explore the functional properties of I_{kr} at a cellular level. So far, the molecular mechanisms identified in mutated KCNH2 channels are very representative among LQTS genes, including haploinsufficiency, dominant negative suppression, altered kinetics and trafficking deficiency [1, 2]. More importantly, the involvement of multiple mechanisms in single KCNH2 channels has been evidenced in an increasing number of studies, being indicative of complicated functional stoichiometries.

The functional effect of stoichiometric property has been examined in a few members of *Shaker*-like potassium channels. In tetrameric complexes constructed from bovine EAG1 and the L322H mutant b-EAG1, the gating properties have been shown to be modulated by independent conformational kinetics of individual subunits[3]. Using the tandem dimer linking two types of drk1 protein subunits with different activation thresholds, Chapman et al. demonstrated that the observed subconductance levels are associated with the activation of one or two subunits in the tetramer [4]. Conversely, little is known about the functional stoichiometry of the KCHN2 channels in the presence of mutation.

Our current work has been to assess the functional implication of the stoichiometric properties of the KCNH2 channel complex. We have developed conformation models for both the homotetrameric KCNH2 and the C-terminus mutants, as well as the heterotetrameric kinetic models for their coexpression, which are further integrated into Luo-Rudy dynamic model in deterministic and stochastic tetramerizations. Our data showed that the functional properties at a cellular level in response to stoichiometric changes due to mutations induced a spectrum of action potential (AP) variations and contributed to the onset of early afterdepolarization (EAD).

2. Methods

2.1. Data processing

Kinetic data were based on wild-type (WT) homomeric KCNH2 channels, homomeric C-terminus mutant channels and the heteromeric protein complex of WT and mutant subunits, expressed in HEK293 [2] cells at room temperature (20 - 22 °C). A potential range of -40 mV to 60 mV was used for activation, -120 mV to -70 mV for deactivation, and -120 mV to 60 mV for inactivation and recovery from inactivation. Trafficking deficiency in the mu-

tant channel proteins and co-expressed channels were evaluated by measuring the signal intensity across cell surface as well as inside the cell based on confocal images. The finalized intensity ratios characterizing the defect of protein transport were the mean values from the 1122fs/147 [5] and the p.Pro872fs [2] mutant proteins.

2.2. Model of stoichiometry

The conformational activity for a single WT or a mutant subunit was characterized by its allosteric coupling process (Figure 1). The voltage sensor transits between a resting and an active state in response to driving forces. The activation gate located at the bottom of S6 segment is conditionally activated, depending on the transduction of S4-S5 linker as a result of electrostatic interaction between individual residues.

The basic subunit stoichiometries were homotetrameric complex formed by either WT or mutant subunits and the 2:2 configurations representing the coassembly of dimeric constructs consisting of one WT and one mutant subunit. The controlled deterministic and stochastic stoichiometries

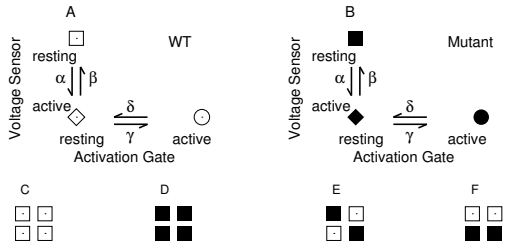

Figure 1. Tetramerization of protein subunits in different stoichiometries.

of specific mutations were established based on the basic variants of stoichiometry and incorporated into virtual tissue model (1D), described by the reaction-diffusion equation,

$$\frac{\partial V}{\partial t} = D\frac{\partial^2 V}{\partial x^2} + \frac{1}{C_m}(I_m + I_s), \quad (1)$$

where V (mV) is the membrane potential, D (cm^2/s) is a diffusion coefficient, I_s is the external stimulating force, and C_m $(\mu F cm^{-2})$ is the membrane capacity.

The characterization of cardiac ion channels and the related cellular physiological properties have been well formulated in the Luo-Rudy AP model, which we used in the present study to explore the functional stoichiometry as well as the interdependence between ion channel proteins in their modulation of AP dynamics.

2.3. Parameter estimation

The functional model for tetrameric complexes were developed by extending the single subunit conformation model to the permutations of four subunits. The conformational kinetics was described as time-homogeneous Markov process based on the stochastic property of single ion channels. The probability that the channel shifts to the state j from the state i after a relaxation time δt is given by

$$P(X(t+\delta t) = X_j)|P(X(t) = X_i) = q_{ij}\delta t + O(\delta t), \quad (2)$$

where $X(t)$ is a random variable representing any energetically stable state of protein rearrangements and q_{ij} specifies the full transition probability from the ith state to the jth state and satisfies the relation,

$$Q := \{q_{ij}\}_{N\times N} = \{k_{ij}\}_{N\times N} \odot \{c_{ij}\}_{N\times N}, \quad (3)$$

where k_{ij} is the transition rate from the ith state to the jth state; c_{ij} is the cooperative factor corresponding to k_{ij}, reflecting intersubunit interactions in a tetrameric complex. The transition rate k_{ij} is exponentially voltage-dependent and formulated as

$$k_{ij} = k_{ij,0} exp(z_{k_{ij}} V \frac{RT}{F}), \quad (4)$$

where $k_{ij,0}$ is the value of rate constant at 0 mV, $z_{k_{ij}}$ is the equivalent charge movement, V is voltage, R is gas constant, and F is Faraday's constant and T is the absolute temperature.

Model parameters were estimated by fitting the tetrameric models to kinetic data. The merit function for data fitting was a nonlinear dynamic system involving matrix exponential,

$$\chi^2 = f(e^{Q(K,C)^T \delta t}, P|_{t-1}). \quad (5)$$

Let E_{kl} denote the $[k,l]$th elementary matrix, the directional derivative $D_{E_{kl}}(exp(A))$ measures the effect of a perturbation in a single entry a_{kl} of a matrix A on the entire matrix exponential and the following relation holds,

$$\frac{\partial e^{At}_{ij}}{\partial A} = \frac{\partial e^{A^T t}}{\partial a_{ij}} = D_{E_{ij}}(t, A^T). \quad (6)$$

Based on Equation 6, the gradients of the merit function was derived as,

$$\nabla\chi^2(K,V) = \sum_{l=1}^{M} \sum_{i,j\in N} D_{P^{[l]}_{ij}}(\delta t, Q^T) : \frac{\partial Q}{\partial k_{ij}}, \quad (7)$$

where $P^{[l]}_{ij}$ is an extended direction matrix consisting of the differences between the probability matrix and the measurement at the time t. Using the augmented block triangular matrix and the power direction derivatives of matrix potential,

$$\begin{bmatrix} e^{Q^T\delta t} & D_{P_{ij}}(\delta t, Q^T) \\ 0 & e^{Q^T\delta t} \end{bmatrix} \quad (8)$$

the matrix exponential and its direction derivatives for parameter search were solved simultaneously.

3. Results

3.1. Conformational changes

A 17-state model was required to reproduce the characteristics of ion channels. Figure 2 shows a heterotetrameric model for a 2:2 configuration, with a loosely cooperative

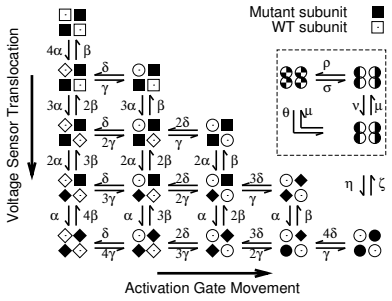

Figure 2. Subunit-based model of co-expressed channels.

transition pathway (allosteric) followed by two concerted transitions. The transition rates of primary allosteric conformation changes are presented in Table 1. The rate con-

Table 1. Rates of primary conformation changes

Parameter of	Model		
Rate Function	Wild-Type	Mutant	Coexpression
α_0	0.2651	0.0970	0.0345
z_α	0.3368	0.4771	0.2538
β_0	0.0045	0.0009	0.0007
z_β	0.3109	0.6689	0.5144
γ_0	0.0151	0.0151	0.0136
z_γ	0.3128	0.3131	0.3455
δ_0	0.0261	0.0239	0.0223
z_δ	0.1164	0.0852	0.0727

stants of voltage sensors at 0 mV for mutants and co-expressed products have one magnitude of reduction compared to the rates for WT.

3.2. Functional effect of stoichiometry

Functional stoichiometry was investigated in both controlled and stochastic tetramerizations and by observing the effect of stoichiometric changes on AP dynamics. The representative results are summarized in Table 2. In comparison with homomeric WT channels, homomeric mutated channels showed a propensity to develop a shorter AP duration. The coexistence of two kinds of homomeric channels in equal amounts resulted in a shorter APD. As a consequence of the interplay between trafficking deficiency and altered kinetics a random tetramerization of WT and mutants resulted in a marked prolongation of APD whereas the coassembly of WT and its mutant in a 2:2 con-

Table 2. Revalance of stoichiometry to APD90.

Stoichiometry	Action Potential Duration (ms)		
of Tetramer	bcl=0.3 (s)	bcl=1 (s)	bcl=1.5 (s)
Homo WT	194.3 ± 6.34	539.6 ± 53.41	608.9 ± 3.86
Homo Mutant[a]	179.1 ± 6.43	556.4 ± 55.17	605.7 ± 2.47
Homo Mutant[ab]	193.4 ± 3.57	565.4 ± 41.84	616.8 ± 5.18
Coexpression[a]	192.9 ± 2.52	560.9 ± 50.10	616.3 ± 4.51
Coexpression[ab]	193.2 ± 1.02	579.5 ± 35.21	627.7 ± 7.49
WT+Mutant[a]	194.6 ± 5.01	560.1 ± 42.98	616.7 ± 5.02
WT+Mutant[ab]	195.2 ± 5.84	551.4 ± 44.35	612.4 ± 4.37
Random Assembly[a]	193.3 ± 1.02	583.8 ± 29.36	631.0 ± 8.52
Random Assembly[ab]	193.2 ± 0.86	583.6 ± 25.89	628.9 ± 8.29

[a] altered kinetics due to mutation
[b] stoichiometric change due to trafficking deficiency

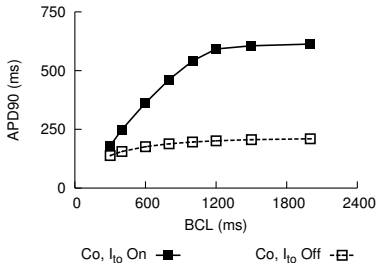

Figure 3. Effect of the expressivity of I_{to} on APD.

figuration generated only an intermediate level of delayed AP repolarization.

To examine the interdependence of I_{kr} and the transient outward k+ channel I_{to}, The conductance of I_{to} was configured to vary from 0.01% to 100% to simulate its different expression levels in epicardium and endocardium. Figure 3 shows the comparison of APD changes in response to changes in the I_{to} expressivity.

3.3. Onset of early afterdepolarization

EAD has been proposed to be one of triggers for polymorphic ventricular tachycardia, Torsade de Points (TdP), in the presence of LQTS. In the current study, a short-long-short (SLS) pacing sequence was introduced after 20 pacing cycles. EADs occurred in the stoichiometries due to kinetic defects and trafficking deficiency (Figure 4A and B). The accumulation of intracellular calcium during the pause induced a significant increase in post-pause calcium transients (Figure 4C and D) and the reactivation of L-type calcium channels I_{lca} were observed in both cases, albeit a small difference in their morphologies (Figure 4E and F).

4. Discussion and conclusions

At present, our knowledge about the functional stoichiometry of I_{kr} complex in the presence of mutations is

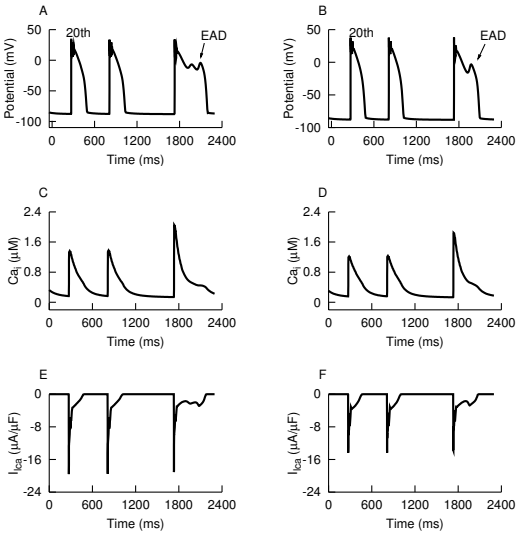

Figure 4. Pause-dependent EAD development. (A) APs with stoichiometry in the presence of kinetic changes. (B) APs with stoichiometry in the presence of altered kinetics and trafficking deficiency. (C) and (D) Intracellular Ca^{2+} dynamics corresponding to A and B, respectively. (E) and (F) L-type Ca^{2+} current density, respectively.

still very limited. The controlled stoichiometry has been used to elucidate the relation of individual subunits to the functional properties in some K^+ channels. The defined stoichiometry based on concatenated constructs from WT and mutant genes, however, may not fully reveal the actual expressions of specific mutants in individual species. Because the expression of the channel proteins follows a strict biogenesis pathway [6], including translation, glycosylation, folding and transportation to cell membranes, the stoichiometry due to mutation may be much complicated than expected. As evidenced in the coexpression of WT KCNH2 and the G628S mutant sunbunits in *Xenopus* oocytes [7] and the WT KCNH2 with the 1122fs/147 mutant counterpart in HEK293 cells [5], the electrophysiological properties don't agree with those predicated from the defined stoichiometry. Our study demonstrated that the subunit-based conformational modeling is a useful alternative for elucidation of a wide spectrum of stoichiometric properties of the KCNH2 proteins. Our experiments showed that the mutation-induced changes in channel stoichiometry affected the AP dynamics and contributed to abnormal AP dynamics, leading to QT prolongation. The data generated predict that the frameshift mutations in the C-terminus of KCNH2 channels are responsible for a moderately severe phenotype, being consistent with the clinical presentations observed in the patients.

The SLS sequence is one of initiating modes preceding TdP in the clinical observation. Our results demonstrated that in this condition, the changes of functional stoichiometry due to mutations may accentuate the APD prolongation and increase the possibility of initiating EADs. The onset of EADs were attributable to marked changes in the intracellular $[Ca^{2+}]_i$ transient and the reactivation of L-type calcium channels. It has been reported that there are transmural gradients in the mRNA expression of both Kv4.3 and KChIP2, the α and β subunits of transient outward potassium I_{to} [8]. Our data showed that the penetrance of the stoichiometry-caused AP abnormality was dependent on the expressivity of I_{to}, indicating that the QT variability may also be affected by the interdependence of individual ion channels.

In conclusion, our study associated the pathophysiological conditions to their underlying stoichiometric properties. These results suggest that stoichiometry of the KCNH2 channel protein complex may be a substrate for variable genetic penetrance and expressivity in long QT syndrome.

References

[1] Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. J Clin Invest 2005;115(8):2018–2024.

[2] Paulussen A, Raes A, Jongbloed RJ, et al. HERG mutation predicts short QT based on channel kinetics but causes long QT by heterotetrameric trafficking deficiency. Cardiovas Res 2005;67(3):467–475.

[3] Schonherr R, Mannuzzu LM, Isacoff EY, Heinemann SH. Conformational switch between slow and fast gating modes - allosteric regulation of voltage sensor mobility in the eag k+ channel. Neuron 2002;35:935–949.

[4] Chapman ML, VanDongen AM. K Channel Subconductance Levels Result from Heteromeric Pore Conformations. J Gen Physiol 2005;126(2):87–103.

[5] Sasano T, Ueda K, Orikabe M, et al. Novel C-terminus frameshift mutation, 1122fs/147, of HERG in LQT2: additional amino acids generated by frameshift cause accelerated inactivation. J Mol Cell Cardiol 2004;37(6):1205–1211.

[6] Caprini M, Ferroni S, Planells-Cases R, Rueda J, et al. Structural Compatibility between the Putative Voltage Sensor of Voltage-gated K+ Channels and the Prokaryotic KcsA Channel. J Biol Chem 2001;276(24):21070–21076.

[7] Sanguinetti MC, Curran ME, Spector PS, et al. Spectrum of herg k+-channel dysfunction in an inherited cardiac arrhythmia. PNAS 1996;93(5):2208–2212.

[8] Zicha S, Xiao L, et al. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. J Physiol 2004;561(3):735–748.

Address for correspondence:

Chong Wang
School of Biomedical Sciences, Universtiy of Ulster
Comorore Road, BTA 56, UK
wang-c1@uslter.ac.uk