Mathematical modelling and kinetic analysis of ion channel subconductance level states

Lijun Shang

Abstract

Mathematical models are proposed to explain the unique subconductance level states in heteromeric Kir4.1/Kir5.1 channels from Xenopustropicalis. The subconductant level states have been confirmed relating to their structure. The modelling results support the co-operatively moving of subunits for the opening of the channel to produce subcontactant level states. It would help to understand the general mechanism of Kir channel gating.

Keywords: ion channel, subconductance states, mathematical modelling

1. Introduction

Ion channels are cell membrane proteins in the body and control the flow of positively charged ions such as sodium and potassium into and out of the cell. An ion channel is traditionally thought to exist in one of two stochastic states i.e. open or closed. However, the electrophysiological recordings on numbers of single ion channels,including several potassium (K+) channels [1-3] have provided evidencesthat ion channel can also experienceintermediate conductance, or 'subconductance', states between its fully open and close states.In Kir channel and in other ion channel in general, these sublevels are theorised to correspond to the movement of the individual subunit which forms the channel [Fig 1]. However, those subconductance states are normally very fast and very difficult to carry out informative quantity kinetics analysis. In a normal homomeric channel the cooperative behaviour between identical subunits means that these events are rapid ,so when viewed using normal timescales, there appears to be a smooth binary transition between open and closed states. Therefore, either new type of ion channel which can show more “measurable” subconductance states, or new methodologies which can complement to the exiting analysis methods to distinguish these “between” states are needed. We have previously shown that heteromeric XTKir4.1/XTK5.1 channels exhibit long-lived subconductancestates,andan ortholog of Kir5.1 from Xenopustropicalis causes a dramatic change in the frequency and duration of these substates;and therefore it can be used as a model system for observing the usually short-lived subconductance levels [3]. Our experiments indicate that in the ‘asymmetric’ Kir4.1-Kir5.1 heteromeric channel these transitions are much slower and allow the sublevels to be seen at normal millisecond timescales. In this paper, we use this channel as an example to explain the subcondctance states in ion channel, and also to build up several mathematical models to compare the analysis on its subconductance kinetics. This would help our understanding of general ion channel gating.

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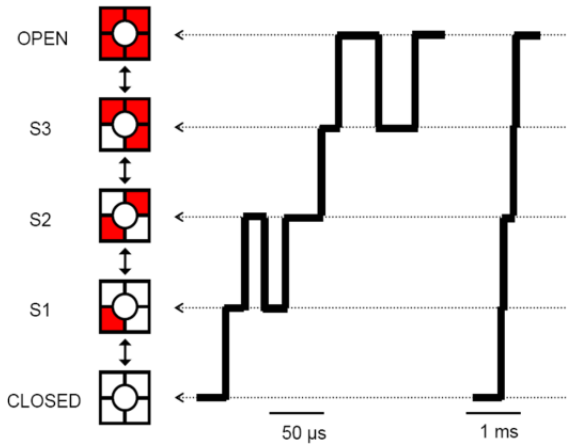

Figure 1: This figure is to explain the possible basis for subconductance states in a K channel. In the closed state where all 4 subunits are closed (white), and there is no current. As each subunit moves from closed to open (red) state the conductance increases to the next sublevel (S1 through to Fully Open S4).

2. Methods

In this study we use the different pieces of available softwaressuch as Clampfit, QuB, HJCFIT, etc [4-6] to detail the kinetic analysis on this model system, based on my experimental data gathered using single channel recording [Fig2], [3].

For the measurement of subconductance states in all type of channels we used the threshold-crossing method, amplitude histograms and HMM (Hidden Markov Model) analysis. Single-channel events were analysed first by ideallyrecording into closed and open dwells, and then fitting histograms of dwell times with mixtures of exponential functions that reflect the dwells in various states using Clampfit 9.2 and HJCFIT software. To ensure the unambiguous detection of brief sublevel events and comparison of sublevel durations we will use QuB analysis software.

A combination of amplitude histograms and dwell time analysis under different analysis software have been compared and contrasted to build a mathematical model to show how many sublevel states exist and formed to gain some insight into its mechanism.

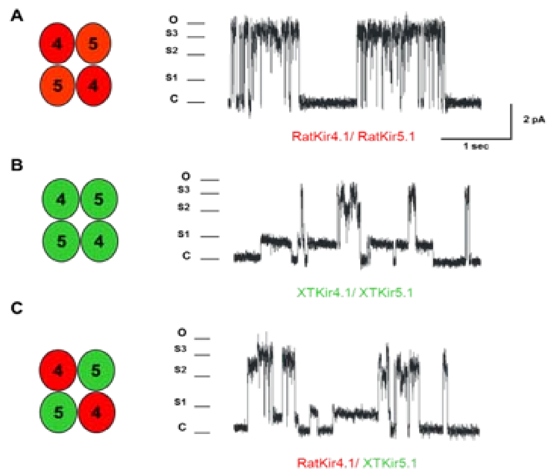

Figure 2: Representative traces of single-channel records of ratKir4.1/ratKir5.1(A), XTKir4.1/XT5.1(B), ratKir4.1/XT5.1(C). No differences exists in either the amplitude of the current or the 'bursting' single channel behaviour with multiple sub-conductance states (A), but the thesubconductance states in XTKir4.1-XTKir5.1 heteromeric channels have a much longer duration. In particular, they have a long S1 sublevel opening which is almost 15 times of the average dwell time duration of the S1 sublevels observed in the rat Kir4.1-Kir5.1 channels (7.5ms vs 0.5ms) (B). ratKir4.1-XTKir5.1 heteromeric channels (C) also exhibit these markedly long S1 sublevels, so itconfirmsthat the XTKir5.1 subunit is responsible for this difference.

3. Results

3.1 Subconductancestates are Separatedby the Normal Analysis

By using logarithmic histogram, the channel can be decided with 5 states: fully open (O), subconductance level 1 (S1), subconductance level 2 (S2),subconductance level 3 (S3), fully close (C). This is agreeable to our experimental analysis by Clampfit [3].

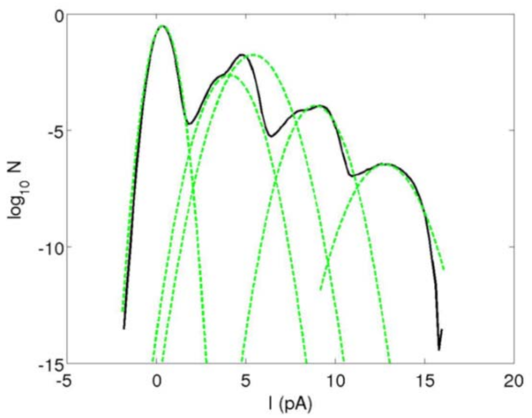

Figure 3: The logarithmic histogram separating five sublevels

3.2 A Linear Gate Scheme is Proposed According to the Structure of Potassium Channel

A linear gate scheme is built according to the structure of potassium channel which has 4 subunit to form the gating pass way. The master equations are therefore built up according to this scheme. By solving these master equations, current values on different subconductance states, probability of each state, and transition rates between all five states are obtained (table 1, 2). They are all agreeable to our experiments data.

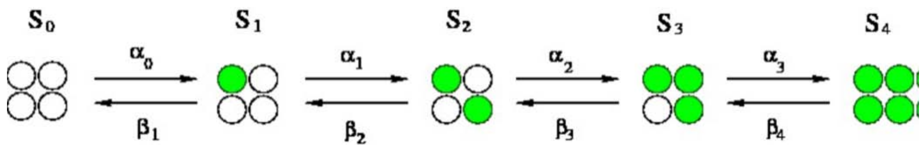

Figure 4: Transitions between all five states, with rates αi, i=0…, 3 and βj, j= 1, …4. and the corresponding master equation that describes the transitions are in the following.

The corresponding master equation that describes the transitions are

$$\dot{P}_0 = \beta_1 P_1 - \alpha_0 P_0, \quad (1.1a)$$
$$\dot{P}_1 = \beta_2 P_2 + \alpha_0 P_0 - (\alpha_1 + \beta_1) P_1, \quad (1.1b)$$
$$\dot{P}_2 = \beta_3 P_3 + \alpha_1 P_1 - (\alpha_2 + \beta_2) P_2, \quad (1.1c)$$
$$\dot{P}_3 = \beta_4 P_4 + \alpha_2 P_2 - (\alpha_3 + \beta_3) P_3, \quad (1.1d)$$
$$\dot{P}_4 = \alpha_3 P_3 - \beta_4 P_4. \quad (1.1e)$$

At steady state (after a long record), the stationary probability at each state satisfies:

$$\hat{P}_0 = \frac{\beta_1\beta_2\beta_3\beta_4}{\alpha_0\alpha_1\alpha_2\alpha_3}\hat{P}_4, \quad \hat{P}_1 = \frac{\beta_2\beta_3\beta_4}{\alpha_1\alpha_2\alpha_3}\hat{P}_4, \quad \hat{P}_2 = \frac{\beta_3\beta_4}{\alpha_2\alpha_3}\hat{P}_4, \quad \hat{P}_3 = \frac{\beta_4}{\alpha_3}\hat{P}_4,$$

and

$$\sum_{i=0}^{4} P_i(t) = 1, \quad \hat{P}_4 = \frac{\alpha_0\,\alpha_1\,\alpha_2\,\alpha_3}{\alpha_0\,\alpha_1\,\alpha_2\,\alpha_3 + \alpha_0\,\alpha_1\,\alpha_2\,\beta_4 + \alpha_0\,\alpha_1\,\beta_3\,\beta_4 + \alpha_0\,\beta_2\,\beta_3\,\beta_4 + \beta_1\,\beta_2\,\beta_3\,\beta_4}$$

Table 1: Current and probability at each state

Sublevel	S_c	S_1	S_2	S_3	S_o
$< I_i >$ (pA)	0.3574	3.4339	5.0317	8.7123	12.7577
$< I_i > - < I_c >$	0	3.0765	4.6743	8.3549	12.4003
$\frac{<I_i>-<I_c>}{<I_o>-<I_c>}$ (%)	0	24.81	37.70	67.38	100
$P(S_i)$ (%)	63.45	11.14	20.88	4.08	0.45

Table 2: Average transition time

Transition	$S_c \to S_1$	$S_1 \to S_2$	$S_2 \to S_3$	$S_3 \to S_o$
N_i	467	12395	409	106
$< \tau_{i,i+1} >$ (s)	0.4697	0.0035	0.0104	0.0078
α_i	2.129	286.28	96.10	128.78
	$S_1 \to S_c$	$S_2 \to S_1$	$S_3 \to S_2$	$S_o \to S_3$
N_i	370	12320	438	106
$< \tau_{j,j-1} >$ (s)	0.0057	0.0061	0.0313	0.0162
β_j	176.53	163.16	31.98	61.69

3.3 A 9-state Model is Proposed with One State for Each Sublevel

According to the structure, each sublevel can also have different states. A 9-state model, named Model 1, is designed to simulate the subunit behaviour of potassium channels. Here, the heteromeric nature of the channel is not considered in this model, but all of the subunits are treated equally.

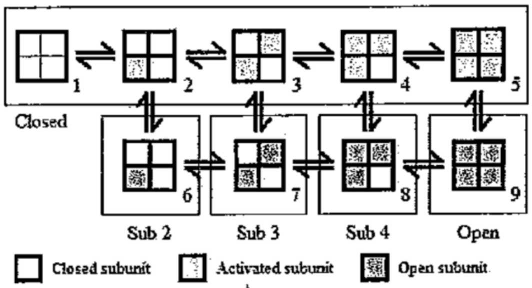

Figure 5: A 9-states model. Grey boxes correspond to activated subunits, where black ones correspond to open subunits. The surrounding boxes mark out the observed current levels.

3.4 A 13-state Model is Proposed with Two States of Each Sublevel

Similarly, a 13-state model, named Model 2, which has two states per sublevel, is considered in Figure 6. This more closely matches the findings of dwell time analysis, so it is expected that the log likelihood of this model will be higher than that of model 1.

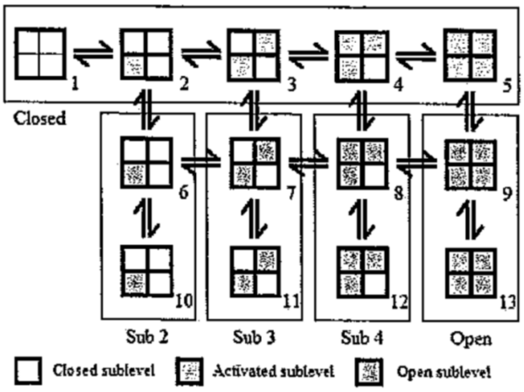

Figure 6: a 13-state model. Grey boxes correspond to activated subunits, where black ones correspond to open subunits. The surrounding boxes mark out the observed current levels.

3.5 Comparisons of Kinetics Parameters on Two Models

We then use QuB software to analyse the channel gating kinetics using the two models as shown above. Mean lifetimes from the real data, and data simulated from two models above areshowed on the below Tables 3. The simulated data consists of twenty segments, each lasting 40000ms. The data is idealised using the Half-Amp method.

Level	Real Data τ (ms)	Model 1 τ (ms)	Model 2 τ (ms)
Closed	2430.51	2601.73	2510.23
Sub 2	26.06	40.70	43.73
Sub 3	8.09	9.97	5.06
Sub 4	51.80	52.30	47.63
Open	16.88	18.28	18.21

Table 4 The exponential (log probability) fits computed using QuB, for both real and simulated data. The statistical errors on the values are huge, due to lack of events and no square-root function, so they have been excluded from this table. The open state was not fitted as there were not enough data points.

Level	Real Data τ (ms)	Model 1 τ (ms)	Model 2 τ (ms)
Closed	2918.57 38.70	2983.71 45.52	3022.29 48.40
Sub 2	110.11 3.86	44.13 0.87	163.96 5.28
Sub 3	148.70 4.37	10.10	1.04
Sub 4	66.89 11.03	49.81	54.73 0.32
Open	20.71	No fit	No fit

4. Discussion

The aim of this study is to use varies software to verify the existing of subconductance states in ion channel. Our results clearly show this. By building mathematical models, it enables us to gain some “inside” knowledge about ion channel gating. Our results are all agreeable to experimental data. In the future, more data are needed for more detailed analysis. It will reduce the statistic errors and provide more conclusive data about the kinetics of each subconductance states. This will further provide details about the structure. Identification of the domains and/or residue(s) responsible for this S1 sublevel duration will also have a major impact on our understanding of the structural basis of the sublevel transitions which occur during channel opening in this and other K+ channels. In particular, by taking advantage of the fact that these particular channels exhibit long-lived subconductance states, we are hoping to better understand the structural basis of subconductance states in heteromeric

Kir4.1/Kir5.1 potassium channels, one of the channels which play an important role in renal tubular transport function, Recent researches have shownthat these channels could be involved in several diseases including type II Bartter syndrome, SeSAME/EAST syndrome, Gitelman-like syndrome [7-9]. Therefore, this study would help us understand which part of the channel is responsible for opening and closing, provide possible structural explanations of how it controls renal tubular transport function, andsuggest potential drug targets in several Kir4.1/Kir5.1 related diseases.

Further on, this study would aid us to understand the role of Kir5.1 in the renal ion transport and associated diseases. Thus it would help us understand which part of the channel is responsible for opening and closing, provide possible structural explanations of how it controls renal tubular transport function, and suggest potential drug targets in several Kir4.1/Kir5.1 related diseases.

Ion channels represent a major percentage of all drug targets, and many drugs are thought to operate by modulating ion channel gating. Identification of the structural elements which control this process may therefore present new therapeutic targets. But all these are beyond the scope of this paper.

5. Conclusion

Ion channel subconductance states have been verified by both experiments and mathematical modelling. However, its gating kinetics still turns to be a very difficult topic. Further more experimental data and more detailed models are needed. Besides, the focus needs to switch to the correlation structure of ion channel and the implication of its kinetics on drug discovery.

Acknowledgment

The author thanks Wan Chen and Alex Gun for their some preliminary works. The work is partly funded by the Physiological Society grant to Dr L Shang. The experiments were conducted at Oxford Centre for Gene Function, Oxford University.

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