A 2-State Markov Model of I_{KACh} based on a Membrane Voltage Dependent Muscarinic M2 Receptor Approach

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Abstract

The heart rate is mediated by the G protein-coupled muscarinic receptor (M2R) activating the acetylcholine (ACh)-dependent K^+ current (I_{KACh}). Here, a novel model for I_{KACh} gating is presented based on recent findings that M2R agonist binding is voltage-sensitive. Furthermore, ACh and pilocarpine (Pilo) manifest opposite voltage-dependent I_{KACh} modulation. In a previous work, a 4-state Markov model of M2R reconstructing the voltage-dependent change in agonist affinity was proposed.

In this work, a 2-state Markov model of I_{KACh} gating purely dependent on the $G_{\beta\gamma}$ concentration is proposed. I_{KACh} is modeled based on the description of Zhang et al. Measurement data are used to parametrize the combined M2R and I_{KACh} model for both ACh and Pilo. The channel model has a linear $G_{\beta\gamma}$ dependent forward and a constant backward rate. For ACh and Pilo, optimal values of model parameters are found reconstructing the measured opposite voltage-dependent change in agonist affinity.

The combined model is able to reconstruct the measured data regarding the agonist and voltage-dependent properties of the M2R-I_{KACh} channel complex. In future studies, this channel will be integrated in a sinus node model to investigate the effect of the channel properties on heart rate.

1. Introduction

The normal heart rate is mediated by the G-protein-coupled, acetylcholine (ACh)-activated inward rectifier K^+ current ($I_{K,ACh}$). $I_{K,ACh}$ can only be seen in the sinus node and atria where it plays a leading role in the regulation of the heart rate via vagal nerve activity. The $I_{K,ACh}$ channel consist of two GIRK1 and two GIRK4 subunits. GIRK subunits can bind $G_{\beta\gamma}$ dissociated from the muscarinic M2 receptor (M2R). The M2R is a G protein-coupled cholinergic receptor that is stimulated by actelycholine and the mushroom poison muscarine. The M2R is sensitive against both transmembrane voltage (V_m) and acetylcholine concentrations, whereas the affinity for acetylcholine concentration itself is voltage sensitive. Ben-Chaim et al. [1] measured that the M2R affinity for ACh decreases upon depolarization. This was shown by charge movement coupled to ACh binding similar to gating currents of voltage-gated ion channels. A structural change in the ligand binding pocket with depolarization caused by structural rearrangements in M2R decreases the affinity for ACh but increases the affinity for a muscarinic poison like pilocarpine (Pilo) [2].

A unique feature of $I_{K,ACh}$ is relaxation. Relaxation refers to an increase of current magnitude with hyperpolarization and decrease with depolarization. This behaviour is due to the voltage dependency of the ligand affinity of the M2R. The M2R binds acetylcholine with higher affinity at hyperpolerized V_m than at depolarized. This results in an increase of $G_{\beta\gamma}$ and therefore in activation of $I_{K,ACh}$. Depolarizarion on the other hand results in deactivation of $I_{K,ACh}$. The characteristic for Pilo is hereby opposite, meaning deactivation of $I_{K,Ach}$ for hyperpolarization and activation for depolarization [2]. The relaxation of $I_{K,ACh}$ can also be seen in Fig. 1. Based on these findings, Moreno-Galindo et al. [3] proposed a 4-state Markov model of M2R reconstructing the voltage-dependent change in agonist affinity.

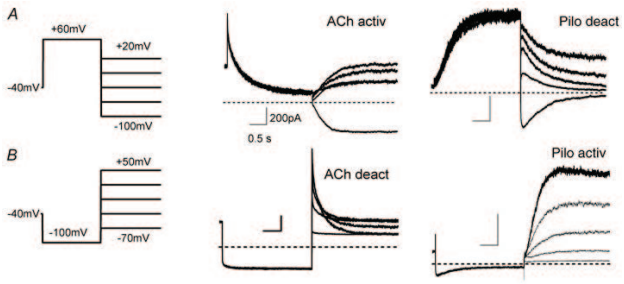

Figure 1. Voltage clamp protocols and resulting I_{KACh} for ACh and Pilo. With a deactivation protocol A), ACh activates the current while Pilo deactivates it. With an activating protocol B) it is vice versa. Fig. adopted from [2].

In this work, a 2-state Markov model of I_{KACh} gating purely dependent on the $G_{\beta\gamma}$ concentration calculated by the 4-state Markov model of M2R is proposed. I_{KACh} itself is modeled based on the description of Zhang et al. [4]. Measurement data [2, 5] are used to parametrize the combined M2R and I_{KACh} model for both ACh and Pilo.

2. Methods

2.1. 4-state Markov model of M2R

The 4-state Markov model of M2R [3] describes the voltage and ligand related changes of the receptorby two voltage states (U1, U2) and two ligand sates (B1, B2) shown in Fig. 2 (left). The states B1+B2 represent the fraction of dissociated $G_{\beta\gamma}$ subunits that can bind to GIRK subunits. The M2R model is described by:

$$\frac{dU1}{dt} = -(\alpha + \gamma \cdot [L]) \cdot U1 + \delta \cdot B1 + \beta \cdot U2, \quad (1)$$

$$\frac{dB1}{dt} = -(K_\alpha + \delta) \cdot B1 + \gamma \cdot [L] \cdot U1 + \beta \cdot B2, \quad (2)$$

$$\frac{dU2}{dt} = -(\beta + K_\gamma \cdot [L]) \cdot U2 + \alpha \cdot U1 + \delta \cdot B2, \quad (3)$$

$$\frac{dB2}{dt} = -(\beta + \delta) \cdot B2 + K_\alpha \cdot B1 + K_\gamma \cdot [L] \cdot U2, \quad (4)$$

with:

$$\alpha = \alpha_0 \cdot exp(z_\alpha \cdot \frac{V_m F}{RT}), \quad (5)$$

$$\beta = \beta_0 \cdot exp(-z_\beta \cdot \frac{V_m F}{RT}), \quad (6)$$

$$K_\alpha = K \cdot \alpha_0 \cdot exp(z_\alpha \cdot \frac{V_m F}{RT}), \quad (7)$$

$$K_\gamma = K \cdot \gamma. \quad (8)$$

α, β, K_α are voltage dependent parameters, γ, δ, K_γ are ligand dependent parameters, $[L]$ is the ligand concentration and the other parameters are constants (see also [3]).

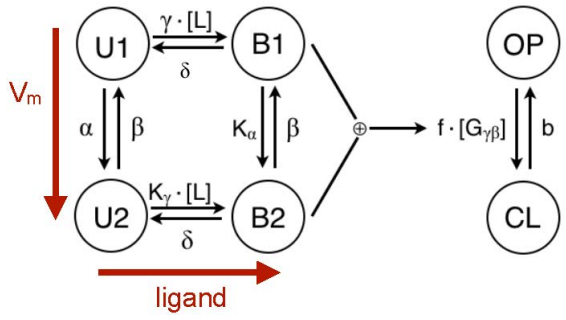

Figure 2. Combined 4-state Markov model of M2R (left) and 2-state model of GIRK channel (right).

2.2. Description of I_{KACh}

I_{KACh} is calculated following Zhang et al. [4]:

$$I_{KACh} = g_{KACh,max} \cdot O_{KACh} \cdot \left(\frac{[K^+]_o}{10\,mM + [K^+]_o}\right) \cdot \cdot \left(\frac{V_m - E_K}{1 + exp\,[(V_m - E_K - 140\,mV)F/2.5RT]}\right) \quad (9)$$

with $[K^+]_o$ the extracellular K^+ concentration, E_K the Nernst potential, $g_{KACh,max}$ the maximum conductance and O_{KACh} the open probability of the channel.

To compare simulated and measured currents for the voltage clamp protocols, both are fitted to a mono-exponential differential equation:

$$I(t) = C_{on,off} + A_{on,off} \cdot e^{\frac{-t}{\tau_{on,off}}} \quad (10)$$

with $C_{on}, A_{on}, \tau_{on}$ being features for activation voltage clamp procols and $C_{off}, A_{off}, \tau_{off}$ features for deactivation protocols. For the ligand protocols, the last values in time of the simulated current for different ligand concentrations are used and compared to the results from [3].

2.3. Adaptation process

An error function (see eqn. **??**) is defined in order to describe the difference between measurement and simulation and to bind states to known values at certain extremes. To optimize the parameters of the model, an iterative scheme of adaptation [6] was applied simultaneously for both voltage [2] (Figs. 3 & 4) and ligand [2, 5] (Fig. 5) protocol data. Based on an initial parameter setup, 10000 random parameter setups were created first. Out of those, the 256 best were selected using the error function and then optimized with the MATLAB function lsqnonlin using the Levenberg-Marquardt algorithm for nonlinear least squares. Based on these optimized setups, 1024 new random setups were created. The optimized setups were then added to the 1024 random setups. Out of those 1290 setups, the 256 best were selected again and optimized. This iteration was done 64 times with the range of random decreasing each iteration.

3. Results

3.1. 2-state model of the GIRK channel

In this work, a 2-state Markov model of GIRK channel gating purely dependent on the $G_{\beta\gamma}$ concentration is proposed (see Fig. 2 (right)). The channel model has a linear $G_{\beta\gamma}$ dependent forward rate f and a constant backward rate b. With $B1 + B2 = G_{\beta\gamma}$, this model is connected

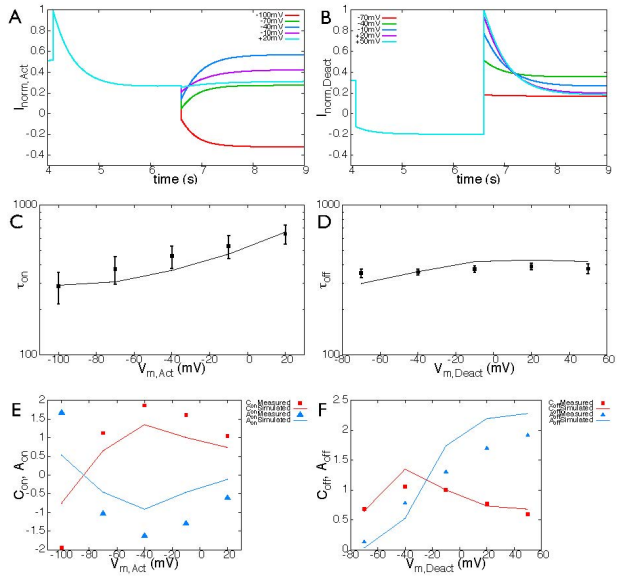

Figure 3. Traces and features of voltage clamp protocols for adaptation with ACh. Dots describe measured data, lines simulated. A) Current during activation and B) deactivation protocol. C) τ_{on}. D) τ_{off}. E) A_{on} and C_{on}. F) A_{off} and C_{off}.

to the 4-state model of M2R. The open probabilty of the 2-state Markov model is given by:

$$\frac{dOP}{dt} = f \cdot [G_{\beta\gamma}] \cdot (1 - OP) - b \cdot OP \tag{11}$$

which results in a relationship with Hill-characteristics between $G_{\beta\gamma}$ and OP. OP is the open probability of GIRK subunits. As there are four independent GIRK subunits in the I_{KACh} channel, the open probability of the whole channel is $O_{KACh} = OP^4$.

3.2. Error function

The relative error for the simulated features in respect to the measured features plus the error added for binding states to specific values is:

$$E = \sqrt{\sum_{i=1}^{n} \left(\frac{\parallel f_{s,i} - f_{m,i} \parallel_2}{\parallel f_{m,i} \parallel_2} \right)^2 + \left((1 - max_{-100mV}(B1 + U1))^2 + (min_{60mV}(B1 + U1))^2 \right) + \left((1 - max_{60mV}(U2))^2 + (1 - max_{-100mV}(U1))^2 + (min_{60mV/-100mV}(Op))^2 \right)_{0\mu M} + \left((1 - max_{60mV}(B2))^2 + (1 - max_{-100mV}(B1))^2 + (1 - min_{60mV/-100mV}(Op))^2 \right)_{10\mu M}}$$

with $f_{m,i}$ being the measured features and $f_{s,i}$ the simulated features. The features are $C_{on}, A_{on}, \tau_{on}, C_{off}, A_{off}, \tau_{off}$ and $I_{norm-X\,mV}$. $I_{norm-X\,mV}$ are the features for ligand protocols at $X = \{-100, -70, -50, 40\} mV$.

Table 1. Resulting parameters for adaptation with ACh

Parameters	Value	Units
z_α	1.47470153466139	[dimensionless]
β_0	945.780854496955	[1/s]
z_β	0.084271236398921	[dimensionless]
γ	14.3607989975767	[1/(s*μM)]
δ	1.88242555566159	[1/s]
K	0.224234357684802	[dimensionless]
f	1.86860617423661	[1/s]
b	0.312804148260665	[1/s]

The component $(1 - max_{-100mV}(B1 + U1))^2 + (min_{60mV}(B1 + U1))^2$ of the error function evaluates the behaviour of the 4-state model at the end of the second voltage step of the voltage clamp protocol. In specific, for the deactivation voltage clamp (Fig. 1, A) the probability of $B1 + U1$ at the end of the second voltage step of -100 mV should be optimally equal to 1. For the activation voltage clamp (Fig. 1, B) this is done so that at the end of the second voltage step of 60 mV the value of $B1 + U1$ should be optimally equal to 0.

The last part of the error function evaluates the behaviour of the 6-state model at two extremes. The extremes are the concentration of ACh for no activation of $I_{K,ACh}$ (0 μM ACh) and the concentration for maximum activation of $I_{K,ACh}$ (10 μM ACh). Meaning that for 0 μM at

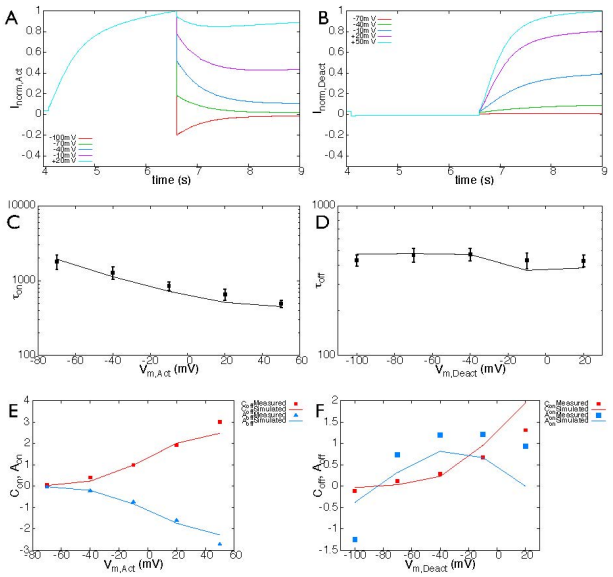

Figure 4. Traces and Features of voltage clamp protocols of adaptation with Pilo. Subfigures as in Fig. 3.

Table 2. Resulting parameters for adaptation with Pilo

Parameters	Value	Units
z_α	0.000601087957322	[dimensionless]
β_0	10641.2881852186	[1/s]
z_β	1.31861879630164	[dimensionless]
γ	0.079336813440797	[1/(s*μM)]
δ	1.23503668763346	[1/s]
K	7.85713760785744	[dimensionless]
f	0.529235846385447	[1/s]
b	0.177621752326039	[1/s]

-60 mV the value of $U2$ and the value of $U1$ for -100 mV should optimally equal to 1. For a concentration of 10 μM analogous the value for $B2$ at -60 mV and $B1$ for 100 mV should be equal to 1. Additionally, the 2-state model is evaluated meaning that OP should be 0 for a concentration of 0 μM ACh and 1 for 10 μM

3.3. Adaptation results

The resulting traces and features for both protocols for ACh can be seen in Fig. 3 and Fig. 5, resp., with the resulting parameters in Table 1. The resulting fit error was 0.835. Analogously, the traces and features for Pilo can be seen in Fig. 4 and Fig. 5 with their adapted parameters in Table 2. Here, the resulting fit error was 0.818.

4. Discussion and conclusion

The combined model is able to reconstruct the measured data regarding the agonist and voltage-dependent properties of the M2R-I_{KACh} channel complex [2, 5]. This is demonstrated by the relatively small fit errors and can visually be inspected in Figs. 3-5 comparing the simulated and measured data. The models mostly lie in the range of the standard deviation of the measured data.

The novelty of this combined 6-state model is, that it describes seperatly the binding of ACh to the receptor and the opening of the GIRK channel. It is additionally new that the voltage-dependency is solely described in the M2R model. All other I_{KACh} models including the one fom Zhang et al. [4] describe the channel itself to have a voltage sensor which can not explain the measurement data presented in Fig. 1.

In future studies, this channel model will be integrated in the sinus node model of Zhang et al. [4] to investigate the effect of the channel properties on heart rate. Further data e.g. of mutations in I_{KACh} should be measured and integrated in the model in order to identify genotype-phenotype relations of these defects regarding heart rate or sinus node disfunction.

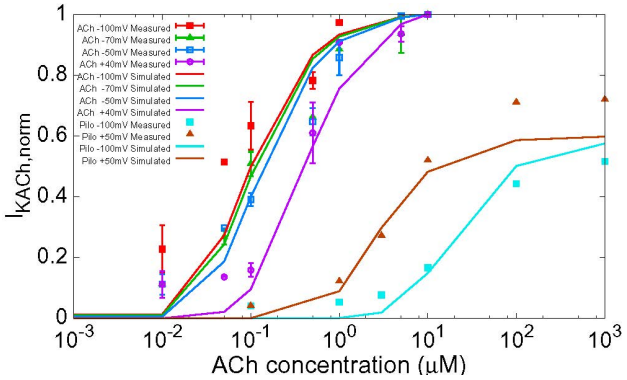

Figure 5. Features of the ligand protocols of adaptation with both protocols (ACh,Pilo). The currents were normalized to $I_{K,ACh}$ elicited by a saturating concentration.

References

[1] Ben-Chaim Y, Tour O, Dascal N, Parnas I, Parnas H. The m2 muscarinic g-protein-coupled receptor is voltage-sensitive. The Journal of Biological Chemistry 2003; 278:22482–22491. ISSN 0021-9258.

[2] Navarro-Polanco RA, Moreno Galindo EG, Ferrer-Villada T, Arias M, Rigby JR, Sanchez-Chapula JA, Tristani-Firouzi M. Conformational changes in the m2 muscarinic receptor induced by membrane voltage and agonist binding. The Journal of Physiology 2011;589:1741–1753. ISSN 0022-3751.

[3] Moreno-Galindo EG, Sanchez-Chapula JA, Sachse FB, Rodriguez-Paredes JA, Tristani-Firouzi M, Navarro-Polanco RA. Relaxation gating of the acetylcholine-activated inward rectifier k+ current is mediated by intrinsic voltage sensitivity of the muscarinic receptor. The Journal of Physiology 2011;589:1755–1767. ISSN 0022-3751.

[4] Zhang H, Holden AV, Kodama I, Honjo H, Lei M, Varghese T, Boyett MR. Mathematical models of action potentials in the periphery and center of the rabbit sinoatrial node. Am J Physiol Heart Circ Physiol 2000;279:H397–H421.

[5] Ben-Chaim Y, Chanda B, Dascal N, Bezanilla F, Parnas I, Parnas H. Movement of 'gating charge' is coupled to ligand binding in a g-protein-coupled receptor. Nature 2006; 444:106–109. ISSN 0028-0836.

[6] Abbruzzese J, Sachse F, Tristani-Firouzi M, Sanguinetti M. Modification of herg1 channel gating by cd2+. The Journal of General Physiology 2010;136:203–224. ISSN 0022-1295.

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