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Electron-conformational transformations govern the temperature dependence of the cardiac ryanodine receptor gating

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Temperature influences many aspects of cardiac excitation-contraction coupling, in particular, hypothermia increases the open probability ($P_{\rm open}$) of cardiac sarcoplasmic reticulum (SR) Ca^{2+}-release channels (ryanodine-sensitive RyR channels) rising the SR Ca^{2+} load in mammalian myocytes. However, to the best of our knowledge, no theoretical models are available for that effect. Traditional Markov chain models do not provide a reasonable molecular mechanistic insight on the origin of the temperature effects. Here in the paper we address a simple physically clear electron-conformational model to describe the RyR gating and argue that a synergetic effect of *external* thermal fluctuation forces (Gaussian–Markovian noise) and *internal* friction via the temperature stimulation/suppression of the open-close RyR tunneling probability can be considered as a main contributor to temperature effects on the RyR gating. Results of the computer modeling allowed us to successfully reproduce all the temperature effects observed for an isolated RyR gating in vitro under reducing the temperature: increase in $P_{\rm open}$ and mean open time without any significant effect on mean closed time.

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Introduction. Sensitive detection and discrimination of temperature cues are fundamental to the survival and prosperity of humans and animals. Hodgkin and Huxley [1] showed more than 60 years ago that temperature could profoundly influence membrane excitability, however, it is in general not well understood how heat affects membrane excitability and how temperature-dependent changes in the activity of ion channels contribute to physiology. A common way to characterize temperature sensitivity is the Q_{10} value (defined as the folds increase in current amplitude upon a 10 °C increase in temperature). For most ion channels, thermal energy affects the rate of conformational transitions by 2-to-5 folds per 10 °C. Among the exceptions are a group of transient receptor potential (TRP) channels with outstanding sensitivity of channel activation to heat. These channels are unique in their ability to directly modify their gating as a function of temperature, being activated at different temperature ranges from noxious cold to noxious heat, and doing so with very large Q_{10} values (~ 30) [2, 3]. How these TRP channels respond to heat with exquisite sensitivity remains mysterious.

From the viewpoint of the condensed matter physics it seems to be absolutely inexplicable how the $\sim 3\,\%$ change in absolute temperature can produce many-fold effect in the channel activity. How can the temperature sensitivity of channel opening be so high? How to explain the apparently counterintuitive behavior of the same temperature sensor giving similar response both upon heating and cooling?

Temperature effects in the heart's cells seem to be not so dramatic as in TRP channels, however, the cells in ventricular myocytes and sinoatrial node (pacemaker) are believed to be one of the most important targets of the temperature efects. Cardiac contractility is profoundly influenced by temperature in all vertebrates. The transient rise in intracellular Ca^{2+} concentration, which underlies excitation-contraction coupling, represents the culmination of many temperature-sensitive cellular processes; i.e. action potential configuration, myofilament Ca^{2+} sensitivity and Q_{10} effects on protein pumps and ion channels.

However, there is a growing evidence that the thermosensitivity of the ryanodine receptors (RyRs) gating [4, 5] can be responsible for the temperature effects on cardiac contractility, in particular, under the quick drops in temperature to near 0 °C, a procedure known as rapid cooling (RC) [5]. Interestingly, that all three RyR isoforms (RyR1, skeletal isoform; RyR2, cardiac isoform; RyR3, brain isoform) respond to RC in a similar manner.

Calcium (Ca^{2+}) release from specific cistern, sarcoplasmic reticulum (SR), through RyR2 channels control muscle contractions and other cell activities [6]. Al-

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tered cardiac RyR's function and hence abnormal calcium releases (sparks) cause different pathologies such as arrhythmia and heart failure [7].

The RC response is most likely caused by a cooling induced conformational change in the RyR, leading to an increase in open probability $P_{\rm open}$ of the channel. To our knowledge, there are yet no biophysically based models of RyR gating which can explain temperature effects. Traditional Markov chains cannot describe the temperature effects because they are purely phenomenological and have no physical basis.

Here, in the Letter, we explore the predictions of simple electron-conformational model (ECM) proposed earlier to describe RyR gating in cardiomyocites and pacemaker cells [8–10], and argue that the model not only can successfully explain the experimentally found temperature behavior of the RyR2 channels [4, 5] but uncovers some universal features of the physiological thermosensitivity. The origin of high thermosensitivity is related with specific quantum tunneling nature of the transition between "open" and "closed" conformations of the RyR channel and unusually low values of the effective temperature that governs the distribution function for its conformational coordinate.

Electron-conformational model. Recently we have demonstrated that the well-known electron-conformational (EC) model (see, e.g., Ref. [11]) is able to capture important features of the individual and cooperative behaviour of the RyRs in ventricular myocytes [8–10]. The EC model of RyR functioning under Ca^{2+} stimuli is based on a biophysical adaptation of the well-known theory of photo-induced structural phase transitions, which has been successfully applied to different solids [12].

The ion-activated RyR channel is a giant ($30 \times \times 30$ nm) macromolecular protein complex comprising 4 subunits of 565 kDa each [6]. The single-particle electron cryomicroscopy studies [13] suggest that the RyR undergoes large rearrangements of structure in both the transmembrane and cytoplasmic segments as it transitions between the closed and open states under Ca^{2+} activation. Existence of at least two (open/close) (meta)stable RyR conformational states is a minimal starting point of any model approach. However, as other ion channels it has a great many of internal electron and conformational degrees of freedom and exhibits remarkable complexities that need to be considered when developing realistic models of ion permeation. Nevertheless, until recently most modelling efforts for RyR channels were focused on a simple "hole in the wall" type model with a set of different (open, closed) states. Our knowledge of molecular mechanisms of RyR channel functioning is limited; hence we are forced to start with the most general "physicists" approach, which implies simplifying biomolecular system to bare essentials with a guidance from experimental data. Modelling the RyR we start with a simple and a little bit naive picture of the massive nanoscopic channel like an elastic rubber tube with a varying cross-section governed by a conformational coordinate Q and a light "electronic" plug switched due to Ca^{2+}-RyR binding/unbinding in the so-called subspace between the RyR and membrane. This electronic plug interacts with the conformational coordinate and acts as a trigger to stimulate its change and related channel cross-section/conductivity. In other words, we reduce a large variety of RyR degrees of freedom to only two: a fast and a slow one, conventionally termed as electronic and conformational one, respectively. Both degrees of freedom are implied to be coupled to realize an EC transformation that is the electronic control of the slow conformational motion. Bearing in mind the main function of RyR channels, we assume only two actual electronic RyR states: "open" and "closed", and a single conformational degree of freedom, Q, described by a classical continuous variable. Change in the electronic and conformational states regulate the main RyR channel function, i.e. determines whether the channel is "open" and permeable for Ca^{2+} ions or "closed" and impermeable to ions. Hereafter we assume that the conformational variable Q specifies the RyR channel "cross-section" or, more precisely, a permeability for Ca^{2+}, that is a conductance, while the dichotomic electronic variable determines its opening and closure. In a full accordance with a "tube" model the RyR conductance we assumed to obey a simple power dependence

$$D(Q) = D\,Q^{\beta}. \tag{1}$$

As a starting point of the EC model algebra we introduce a simple effective Hamiltonian for a single RyR channel as follows [8–10]

$$H_s = -\Delta\hat{s}_z - h\hat{s}_x - pQ + \frac{K}{2}Q^2 + aQ\hat{s}_z, \tag{2}$$

where s_z and s_x are well-known Pauli matrices, and the first term describes the bare energy splitting of "up" and "down" (electronically "open" and "closed") states with an energy gap Δ, while the second term describes a quantum mixing effect. The third, linear in Q term formally corresponds to the energy of an external conformational stress, described by an effective stress parameter p. The fourth term in (2) implies a simple harmonic approximation for the conformational energy, K is the effective "elastic" constant. The last term describes the

EC interaction with the coupling parameter a. Hereafter we make use of the dimensionless conformational variable Q; therefore all of the model parameters (Δ, h, p, K, a) are assigned energy units. Two eigenvalues of our Hamiltonian

$$E_{\pm}(Q) = \frac{K}{2}Q^2 - pQ \pm \frac{1}{2}\left[(\Delta - aQ)^2 + h^2\right]^{1/2} \qquad (3)$$

define two branches of the conformational potential (CP), attributed to electronically closed (E_-) and electronically open (E_+) states of the RyR, respectively. Given $h = 0$ we arrive at two diabatic potentials $E_{\pm}(Q)$ for electronically closed and open states, shown in Fig. 1 at $\Delta = 0$. Two minima are separated by an intersection

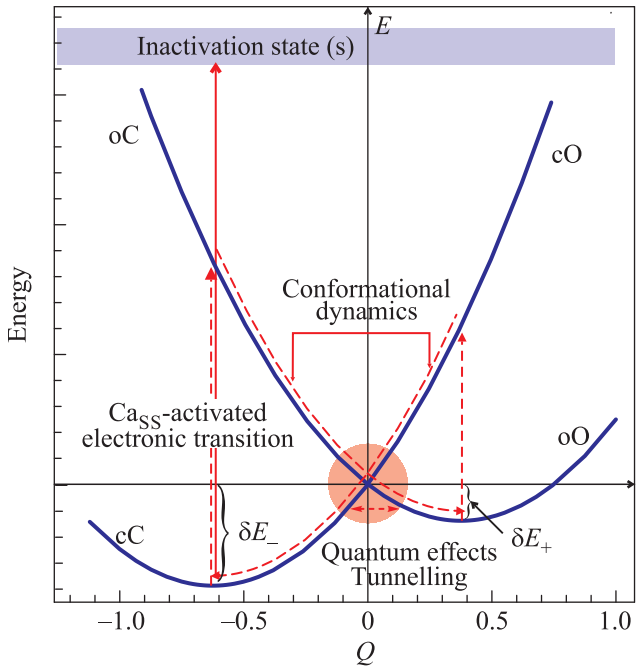

Fig. 1. Conformational potential in the EC-model of the RyR2 channel given $h = 0$ with a global minimum for a closed state. Small letters "c, o" are used for electronically closed and open states, respectively, and capital letters "C, O" for conformationally closed and open states, respectively. The energy separations δE_-, δE_+ are the potential barriers seen from the bottom of two diabatic potentials, respectively. Vertical arrows point to Ca^{2+}-induced Franck–Condon (FC) electronic transitions, horizontal arrow points to a non-FC tunneling transition, downhill arrows point to a conformational dynamics. In addition to original EC-model we have introduced an excited inactivation state

point at $Q = 0$. The effective stress parameter p is assumed to depend on the calcium concentrations at *trans* face of the channel [8]. Rise of p in the interval $(-1, +1)$ leads to a crucial modification of CP from that of stabilizing closed RyR state to that of stabilizing open RyR state.

The classical dynamics of the conformational coordinate we assume to obey a conventional Langevin equation of motion [14]:

$$M\ddot{Q} = -\frac{\partial}{\partial Q}E(Q) - M\,\Gamma\dot{Q} + \gamma\sqrt{T}\,R(t), \qquad (4)$$

where first term describes a total systematic conformational force with M being an effective RyR mass (below M let to be unity), Γ is an effective dimensionless "internal" friction constant. The last term describes the thermal fluctuation force (Gaussian–Markovian noise), γ is a random force parameter, T is a temperature, $R(t)$ is a delta-correlated stationary Gaussian process with a zero-mean value. It is of a principal importance to note that we cannot expect here the direct relation between the effective systematic frictional force and the random force as it is dictated by the fluctuation-dissipation theorem [14]. The random force determines an "external" friction. It is worth noting that a detailed mathematical formulation of the electron-conformational model is performed in PhD thesis by M. Philipiev [15].

Hereafter we neglect the temperature dependence of the main EC-model parameters except the friction parameter Γ and the random force parameter γ, which are assumed to obey the Arrhenius type temperature dependence:

$$\Gamma(T) = \Gamma_0 e^{E_\Gamma/k_B T}; \quad \gamma(T) = \gamma_0 e^{E_\gamma/k_B T} \qquad (5)$$

with activation energies E_Γ and E_γ, respectively.

Hereafter, we shall consider the quantum mixing parameter h to be negligibly small for large nanoscopic RyR channel. Nevertheless, we shall take into account quantum tunneling transitions between the two diabatic potentials (see Fig. 1). We assumed a resonant quantum tunneling takes place between two branches of the conformational potential with the probability obeyed the effective Gamov law as follows

$$P_{\rm tun} = P_0 e^{-A_{\rm tun}\Delta Q\sqrt{\Delta E}}, \qquad (6)$$

where ΔQ is the width, ΔE is the height of the energy barrier, or the energy separation between the tunneling points and the intersection point (see Fig. 1), and P_0 is an effective tunneling attempt frequency. Obviously, a sizeable tunneling probability will be observed near the intersection point where both ΔE and ΔQ turn into zero. It is precisely this fact that can result in a strong temperature effects for the RyR channel gating, if we

take into account the distribution functions for the conformational coordinates in diabatic potentials [15]:

$$\rho(Q) = \frac{1}{\sqrt{2\pi}\sigma} e^{-(Q-Q_0)^2/2\sigma^2} = \frac{1}{\sqrt{2\pi}\sigma} e^{-E(Q)/k_B T^*}, \tag{7}$$

where $\sigma^2 = \gamma^2 TM/(2K\Gamma)$, Q_0 is the conformational coordinate in the CP minima, $E(Q) = 0.5KQ^2$ is elastic energy, $T^* = \frac{\gamma^2}{2\Gamma} T$ is an effective temperature which strictly coincides with real temperature: $T^* = T$, if the Langevin dynamics obeys the fluctuation-dissipation theorem with $\gamma^2 = 2\Gamma$ [14]. Given $E_\Gamma > 2E_\gamma$ the effective temperature can be much less than the real temperature thus dramatically amplifying the relative temperature effect. Increasing the temperature we arrive at a rise in the occupation of the conformational states near the intersection point thus increasing the probability of the open-close tunneling transition. Lowering the temperature leads to a quenching of the conformational coordinate near the minima with a negligible open-close tunneling transition probability. We see that the features of the tunneling transitions between CP branches together with the concept of the effective temperature can provide a qualitative explanation of the main temperature effects on the RyR gating. At present we cannot perform a full quantitative description of the temperature effects. Indeed, despite physical clearness and visible simplicity the EC model does include more than ten parameters whose values and temperature dependencies are not well established. From the other hand, the experimental data available does not provide a sound basis for the model fitting. Nevertheless, we can demonstrate that the EC model is able to explain experimental data available given reasonable values of the EC model parameters. As a starting point for the simulation procedure we address an analytical expression for the temperature dependencies of the integrated tunneling transition probabilities $\langle P_{\rm tun}\rangle$ for the open-close and close-open transitions [15]:

$$\langle P_{\rm tun}\rangle = P_0 \int_{-\infty}^{+\infty} e^{-A_{\rm tun}\Delta Q\sqrt{\Delta E}} \frac{e^{-(Q-Q_0)^2/2\sigma^2}}{\sqrt{2\pi}\sigma} dQ. \tag{8}$$

As a result we arrive at curves with an abrupt rise of $\langle P_{\rm tun}\rangle$ when T^* approaches to $T^*_\pm = \frac{1}{k_B}\delta E_\pm$, a maximum close to $T^*_\pm$ and a slow fall at $T > T^*_\pm$. Obviously, we should choose the parameters of the EC model so that $T^*_\pm$ were close to physiological temperatures. For illustration we present in Fig. 2 the temperature dependencies of $\langle P_{\rm tun}\rangle$ calculated given typical values of the main EC-model parameters [8–10]: $\Delta = h = 0$, $a = 5$, $K = 10$, and $P_0 = 25\,\mathrm{ms}^{-1}$, $A_{\rm tun} = 150$, and $\Gamma_0 = 4.3 \cdot 10^{-12}$, $E_\Gamma = 666\,\mathrm{meV}$ (15.2 kcal/mol), $\gamma_0 = 3.1 \cdot 10^{-3}$, $E_\gamma = 25.6\,\mathrm{meV}$ (0.6 kcal/mol) were chosen

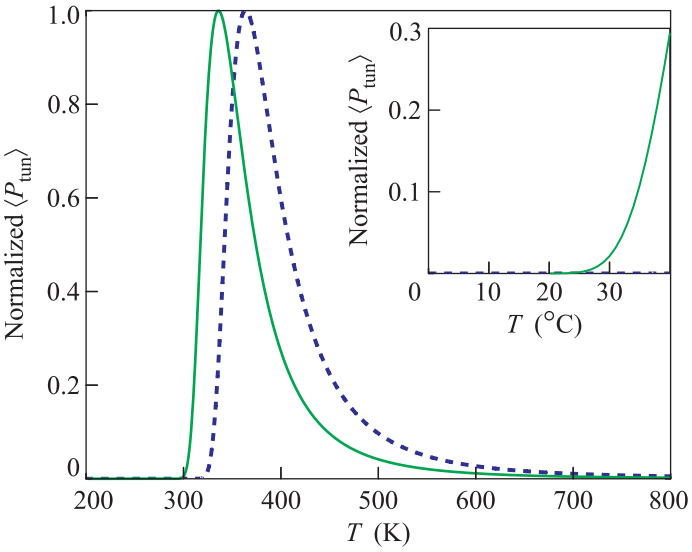

Fig. 2. Temperature dependence of the tunneling open-close (solid curve) and close-open (dotted curve) probabilities. Inset shows the physiological temperature range in a large scale

here for a wide temperature range far past the boundaries of physiological temperatures. As anticipated for the EC model with a global minimum for closed state ($\delta E_- > \delta E_+$) the sharp rise of the open-close tunneling transition probability near the physiological temperatures is shifted to lower temperatures as compared with that for the close-open transition. It is worth noting that in the range of physiological temperatures 30–40 °C the Q_{10} coefficient for the open-close tunneling probability amounts to $Q_{10} = 14$ while for the close-open tunneling probability Q_{10} is negligibly small (see insert in Fig. 2). It is worth noting some similarities between our approach and standard Kramers calculation of rate coefficients for thermoactivated reactions [16].

Results and discussion. As we claimed above, our aim is to study modification of cardiac RyR gating and conductance by temperature in terms of the EC model and explain basic experimental data by R. Sitsapesan et al. [4].

Making use of a standard ECM technique [8] we have performed a series of computer simulations of the RyR gating taking into account the Langevin conformational dynamics in diabatic potentials $E_\pm(Q)$, fast Ca^{2+} activated Franck–Condon electronic transitions cC → oC and oO → cO with probabilities $P(\mathrm{cC} \to \mathrm{oC}) = 0.028\,\mathrm{ms}^{-1}$, $P(\mathrm{oO} \to \mathrm{cO}) = 0.001\,\mathrm{ms}^{-1}$, respectively, the stress parameter $p = -1$, and other parameters as recited above. All the parameters are chosen to provide a matching with the experimental data [4] by eye alone. The simulation results are shown in Figs. 3–6 where these are compared with the experimental data [4].

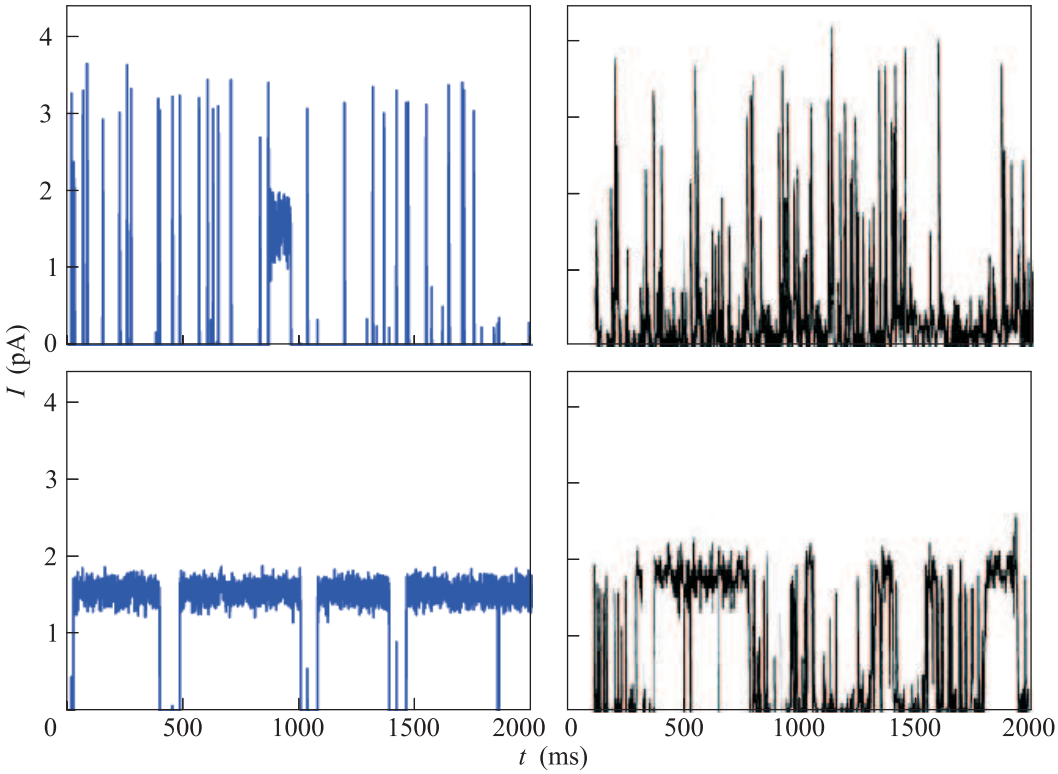

Fig. 3. Representative current fluctuations from a single RyR2 at 23 °C (upper panels) and 5 °C (bottom panels). Left panel: the results of the ECM simulations, right panels: the data taken from [4]

Fig. 3, right panel, shows representative current fluctuations from a single sheep cardiac RyR channel in the lipid bilayer activated with 10 μM calcium at the cytosolic ("cis") face of the channel at two temperatures, 23 and 5 °C. Cooling from 23 to 5 °C strongly modifies the RyR2 gating by essentially decreasing maximal conductance (see also Fig. 4) and increasing the channel open probability P_{open} from 0.06 to 0.75 (see also Fig. 5). Kinetic analysis of the open and closed lifetime distributions clearly demonstrated that the underlying cause for the increase in P_{open} is an increase in open lifetime duration, while no significant effect on closed lifetime duration was observed (see Fig. 6). The conductance of the sheep cardiac RyR channel (see Fig. 4) is decreased as the temperature is reduced with $Q_{10} \approx 1.5$ obtained between 10 and 20 °C.

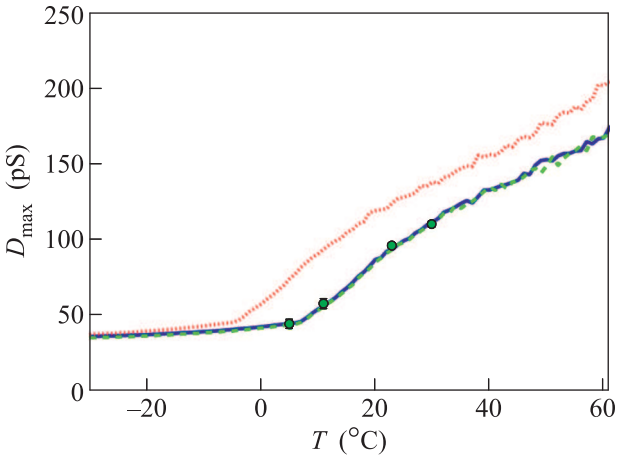

Fig. 4. Temperature dependence of the RyR maximal conductance: the points are experimental data [4], curves are the EC model simulation (see text for details)

We see that the EC model has the power to explain all the main features of the temperature effects on the RyR gating with the same set of the model parameters. Indeed, rather moderate cooling from 23 °C to 5 °C can strongly modify the RyR2 gating by increasing open times and decreasing maximal conductance. Simple visual comparison of the data shown in left and right panels in Fig. 3 points out that there is more than a simple qualitative matching between theory and experiment, especially considering that at variance with our simulation procedure the data [4] collected at all temperatures were filtered so that lifetimes with durations of less than 2 ms were not fully resolved.

Further validation of large explanatory and predictive possibilities of the EC model we see in Figs. 4–6,

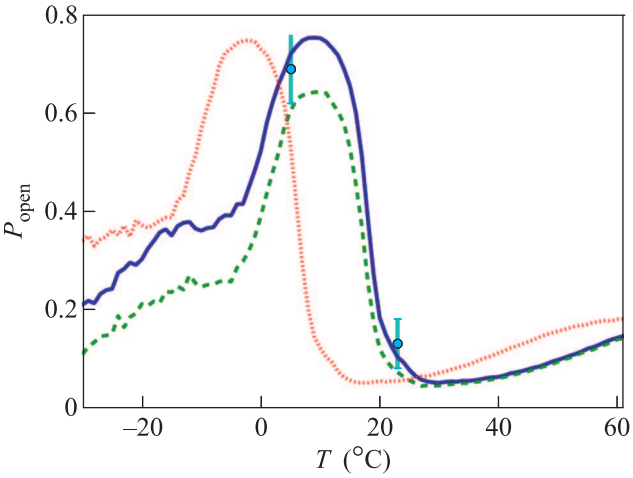

Fig. 5. Temperature dependence of the P_{open} given different values of the activation energy E_Γ and the ECM coupling constant. The points are experimental data [4], curves are the EC model simulation (see text for details)

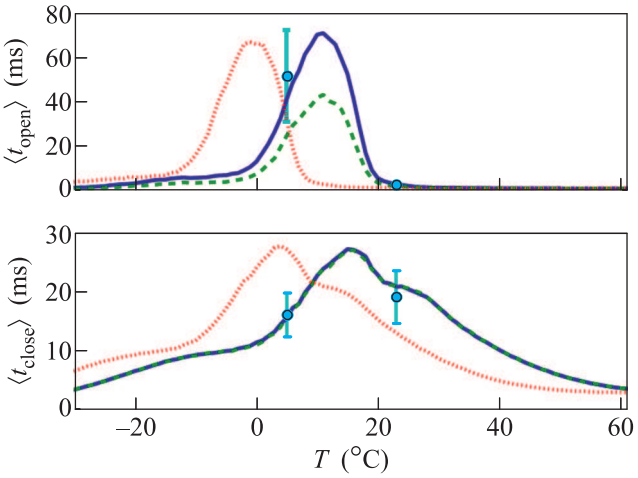

Fig. 6. Temperature dependence of $\langle t_{open}\rangle$ and $\langle t_{close}\rangle$: the points are experimental data [4], curves are the EC model simulation (see text for details)

where simulated temperature dependencies of the maximal conductance, open time probability, P_{open}, mean open and mean closed times, $\langle t_{open}\rangle$ and $\langle t_{close}\rangle$, are compared with experimental data by R. Sitsapesan et al. [4]. Main (solid) curves in these figures are calculated for the EC parameters given above in text; dotted curves are calculated for the same set of parameters except E_Γ lowered by 4 %; dash-dotted curves are calculated for the same set of parameters except the electron-conformational coupling constant a lowered by 2 %.

Given the conductance parameter value $D = 120$ and $\beta = 0.65$ we arrive at a nice quantitative description of the temperature dependence of maximal conductance which rather sharply falls with cooling (Fig. 4). It is clearly seen that in the temperature range from −20 to +20−25 °C the RyR gating is very temperature sensitive, P_{open}, $\langle t_{open}\rangle$, and $\langle t_{close}\rangle$ show a very strong nonlinear temperature dependence with Q_{10} up to extremely large values of $Q_{10} \approx 20$ in the temperature range 15–20 °C. Obviously, P_{open} decreases rapidly with increasing the temperature up to +20−25 °C mainly due to a rapid decrease of the mean open time $\langle t_{open}\rangle$, while the mean closed time $\langle t_{close}\rangle$ doesn't change significantly. However, with a further increase of the temperature the RyR gating becomes less temperature sensitive.

Interestingly, a minor (4 %) decrease in the activation energy for "internal" friction results in a sizeable (10–15 °C) shift of all the curves in Figs. 4–6 to low temperatures, while a 2 % decrease in the electron-conformational coupling constant a leads only to an amplitude reduction, negligible for maximal conductance and $\langle t_{close}\rangle$, however, sizeable for P_{open} and, particularly, for $\langle t_{open}\rangle$. Unfortunately, at present we cannot perform a regular parameter analysis of the model because of experimental data are limited or not available.

In summary, we have demonstrated that simple physically clear electron-conformational model can provide not only qualitative, but also convincing quantitative explanation of the temperature effects on the RyR gating. Synergetic effect of *external* thermal fluctuation forces (Gaussian–Markovian noise) and *internal* friction via the temperature stimulation/suppression of the open-close RyR tunneling probability can be considered as a main contributor to the temperature effects. Results of the computer modeling allowed us to successfully reproduce all the temperature effects observed for an isolated RyR gating in vitro under reducing the temperature [4]: increase in P_{open} and mean open time without any significant effect on mean closed time. We can conjecture that the temperature dependence of the "internal" and "external" frictions and a specific thermosensitivity of the probability of the quantum tunneling transition between two protein conformations can explain outstanding temperature sensitivity of the thermo-transient receptor potential (thermo-TRP) channels that constitute the molecular basis for the detection of changes in ambient temperature by sensory neurons in animals [2, 3].

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