# GATING MECHANISMS OF THE SARCOPLASMIC RETICULUM CALCIUM CHANNEL

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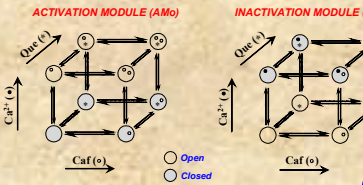
## ABSTRACT

*We have developed a model of the ryanodine receptor – the calcium channel of the sarcoplasmic reticulum. The model accurately describes available experimental data on the channel activity at various concentrations of calcium ions, caffeine and quercetin. The proposed mechanism is the allosteric regulation of calcium ion affinity by both caffeine and quercetin, and the existence of two independent gates controlled by ligand binding to the receptor. By fitting the data we are able to derive binding affinities and Hill coefficients of the ligands, and explain why quercetin is an activating agent stronger than caffeine, or how caffeine and quercetin can activate the channel at very low $Ca^{2+}$ concentration (~$10^{-11}$ M). We predict that the activation regime at saturating caffeine or quercetin should present four distinct regions at increasing $Ca^{2+}$, corresponding to the successive increase in the open probability due to each monomer of the receptor. Another interesting prediction is the enlargement of the activity domain toward higher $Ca^{2+}$ concentrations in the presence of caffeine or quercetin.*

## THE MODEL




- *2 independent gates*
- *Allosteric regulation by*
  - $Ca^{2+}$
  - Caffeine (Caf)
  - Quercetin (Que)

➢ $Ca^{2+}$ bound to AMo opens the channel
➢ $Ca^{2+}$ bound to IMo shuts the channel

*The ryanodine receptor (RyR) has 4 monomers*

$$P_o = P_A \times P_I = 0.25\,(P_A^{m1} + P_A^{m2} + P_A^{m3} + P_A^{m4}) \times P_I$$

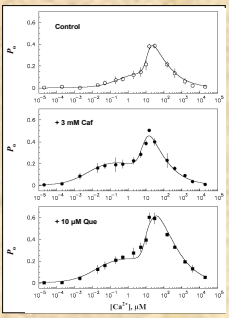
***Steady state open probability*** ($P_o$)

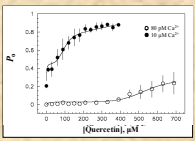
**Theoretical calculation**

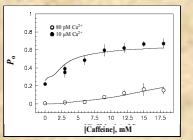
- First order kinetics for binding reactions
- Mass balance equations
- Thermodynamical equilibrium constraints

## RESULTS

***There is a very good agreement with available experimental data (Lee et al., 2002)***




***PARAMETERS OBTAINED***

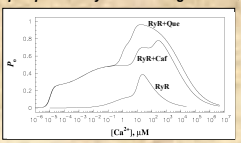
- Dissociation constants (*K*)
- Hill coefficients (*h*)

| *MODULE / monomer* | $Ca^{2+}$ | | | L = Caf | | |
|---|---|---|---|---|---|---|
| | $K_0$ (μM) | *h* | *K* (μM) | $K_L$ (μM) | $h_L$ | $K_{L0}$ (μM) |
| ***ACTIVATION*** | | | | | | |
| *monomer 1* | 0,8 | 0,65 | 0,002 | 1500 | 4 | 3971 |
| *monomer 2* | 13 | 3 | 7,9 | 200 | 2 | 422 |
| *monomer 3* | 11 | 3,5 | 0,00001 | 5500 | 2 | 2,1E+14 |
| *monomer 4* | 13 | 3 | 170 | 50000 | 2 | 1057 |
| ***INACTIVATION*** | | | | | | |
| *all monomers* | 15 | 0,55 | 4000 | 6000 | 3 | 2155 |

| *MODULE / monomer* | $Ca^{2+}$ | | | L = Que | | |
|---|---|---|---|---|---|---|
| | $K_0$ (μM) | *h* | *K* (μM) | $K_L$ (μM) | $h_L$ | $K_{L0}$ (μM) |
| ***ACTIVATION*** | | | | | | |
| *monomer 1* | 0,8 | 0,65 | 0,002 | 1,5 | 2 | 10,5 |
| *monomer 2* | 13 | 3 | 0,5 | 0,5 | 1 | 8788 |
| *monomer 3* | 11 | 3,5 | 0,00001 | 300 | 4 | 5,8E+07 |
| *monomer 4* | 13 | 3 | 5 | 0,6 | 1 | 10,5 |
| ***INACTIVATION*** | | | | | | |
| *all monomers* | 15 | 0,55 | 10000 | 210 | 4 | 85,9 |

## PREDICTION

***Open probability at saturating Caf or Que***




## DISCUSSION

- *Ligand binding sites in activation module have different properties among monomers*
- *Ligand binding sites in inactivation module are similar among monomers*
- *A single monomer can access the gate sensor coupled to AMo*
- *The choice of this monomer is stochastic*
- *Quercetin is a more powerful activator of the RyR receptor than caffeine*
- *The channel is activated in 4 successive domains at saturating Que or Caf*

## REFERENCES (selective)

1. Baran I., *Biophys. J.* 84: 1470-1485 (2003)
2. Baran I., *Biophys. J.* 89: 979-998 (2005)
3. Lee E.H. et al., *Biophys. J.* 82: 1266-1277 (2002)

## ACKNOWLEDGEMENTS

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