Effect of Ca^{2+} Gradient on the Structure of Sarcoplasmic Reticulum Membranes

O. M. Vekshina, Yu. A. Kim*, and N. L. Vekshin*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 11, pp. 517-521, November, 2007
Original article submitted September 8, 2006

The structure of sarcoplasmic reticulum membranes was studied in the presence of modeled transmembrane Ca^{2+} gradient corresponding to the status of Ca^{2+} depot at different stages of the muscle contraction—relaxation cycle in health and disease. Various sites of the membrane were characterized using spectral analysis of tryptophan, pyrene, and merocyanine-540 fluorescence without evaluating specific changes in the molecules of membrane components (Ca^{2+}-ATPase, ryanodine receptor, and lipids). The transmembrane Ca^{2+} gradient modulates the protein-lipid interactions and structural characteristics of the membrane. The proposed model can be used for studies of the effects of pharmacologically active substances and endogenous regulators.

Key Words: *sarcoplasmic reticulum; Ca^{2+} concentration gradient; protein-lipid interactions; fluorescence*

The possibilities of adaptation of the regulatory mechanisms of the muscle tissue to hyperexercise and prevention of the pathological processes are important problems of modern biology. We studied membrane structure of the sarcoplasmic reticulum (SR), the main Ca^{2+} depot in skeletal muscle cells, on a model simulating normal exercise and Ca^{2+} exhaustion and overload; this model can be used for testing of endogenous and exogenous bioactive substances.

Ryanodin and caffeine are exogenous stimulators of Ca^{2+} channel isolated from plants, sphingolipids are endogenous stimulators [1]. The function of ryanodine receptor is regulated by satellite proteins: luminal, transmembrane, and cytoplasmic (calsequestrin, triadin, junctin, *etc.*) [1-3] activated in the presence of certain ratios of cytoplasmic ($[Ca^{2+}]_{cyt}$) and SR concentration of Ca^{2+} ($[Ca^{2+}]_{SR}$). Transmembrane Ca^{2+} concentration gradient ($[Ca^{2+}]_{SR}/[Ca^{2+}]_{cyt}$) is maintained by the work of ryanodine receptor and Ca^{2+}-ATPase and is the key factor regulating the functions of all components of the SR Ca^{2+} depot [4]. $[Ca^{2+}]_{SR}$ fluctuate within a wide range due to Ca^{2+}-ATPase work during Ca^{2+} absorption as a result of Ca^{2+}-ATPase and luminal proteins interactions [5]. The interaction between ryanodine receptor and Ca^{2+}-ATPase in the Ca^{2+} depot system is a well coordinated process; according to experimentally validated scheme [6], the phase of Ca^{2+} release is replaced by the phase of Ca^{2+} pumping back into SR only at mean $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{SR}$ values. When Ca^{2+} depot is overloaded or unloaded, Ca^{2+} is only released and only absorbed, respectively.

We studied structural changes in SR membrane depending on transmembrane gradient of Ca^{2+} ions corresponding to certain states of Ca^{2+} depot at different stages of muscle contraction—relaxation cycle under normal and extreme conditions.

MATERIALS AND METHODS

All SR compartments of rabbit white skeletal muscles were studied. Heavy fraction of SR fragments was isolated from the terminal cisterns, which re-

N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow; *Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** olgavek@yandex.ru. O. M. Vekshina

0007-4888/07/1445-0677

lease Ca^{2+} through the ryanodine receptor Ca^{2+} channel in response to stimulation. Two subfractions of the light fraction were isolated from long tubules: caffeine-sensitive (functionally similar to heavy fraction, but not contaminated with mitochondria) and caffeine-insensitive (Ca^{2+} uptake only).

Fractions of fragmented SR (FSR) were isolated as described previously [7]. The concentration gradient was created by passive loading of FSR vesicles in a medium containing different concentrations of Ca^{2+} and subsequent binding of extravesicular Ca^{2+} ions. The choice of concentrations was determined by physiologically significant values. The mean value (1 mM) corresponded to SR Ca^{2+} depot status at rest, *i.e.* when Ca^{2+} release and uptake are absent, but the system can be rapidly triggered by stimulation of Ca^{2+} release through the ryanodine receptors. The release is followed by Ca^{2+} uptake due to rapid activation of Ca^{2+}-ATPase by released Ca^{2+} ions. The extreme values of the gradient are exhaustion and overload of Ca^{2+} depot. FSR (70 μg/ml) was passively loaded with Ca^{2+} (0.3, 1.0, 3.0 mM $CaCl_2$) in 0.1 M KCl, 20 mM HEPES (pH 7.1) for 4 h at 24°C directly before fluorescence measuring (Perkin-Elmer Coleman Luminescence spectrometer). Extravesicular Ca^{2+} was bound by adding EGTA excess (20 mM). Pyrene and merocyanine-540 were added to the cuvette to concentrations of 0.8×10^{-6} and 10^{-6} M, respectively. The fluorescence of tryptophan (Trp) was measured at λ_{ex}=286 nm (3-nm aperture) and λ_{em}=345 nm (4-nm aperture); pyrene at λ_{ex}=336 nm (2-nm aperture), λ_{em}=395 nm (for monomers), and λ_{em}=480 nm (for eximers; 4-nm aperture); merocyanine-540 at λ_{em}=520 nm (4-nm aperture) and λ_{em}=550-630 nm (8-nm aperture), maximum λ_{em}=580 nm. The dilution effect was taken into consideration. Shielding and reabsorption were negligible.

RESULTS

Spectral characteristics of three FSR fractions were similar, all Trp residues were in hydrophobic environment, primarily at the protein-lipid interface [7]; they anchored protein in the membrane [8]. The main Trp signal originated from the Ca^{2+}-ATPase molecule, because the content of other integral proteins (for example, ryanodine receptor) was negligible even in the heavy fraction in comparison with Ca^{2+}-ATPase [9]. That is why Trp fluorescence characterizes mainly Ca^{2+}-ATPase and its interactions with lipids.

The intensity of Trp fluorescence varied significantly, depending on Ca^{2+} loading (Fig. 1, *a*), but the fluorescence maximum did not change (335 nm), which means that Trp residues remained in the hydrophobic environment. The minimum intensity was observed at 1 mM Ca^{2+}. Hence, the conditions for protein-lipid interactions of Ca^{2+}-ATPase considerably depended on changes in Ca^{2+} gradient. This is also confirmed by the type of relationship between Ca^{2+} gradient and pyrene quenching of Trp fluorescence (Fig. 1, *b*): pyrene directly interacting with Ca^{2+}-ATPase molecule quenches mainly the fluorescence of Trp residues facing the lipid phase [8]. The minimum quenching of Trp fluorescence was observed at 1 mM Ca^{2+}. Availability of Trp on the lipid side is reduced at this concentration of

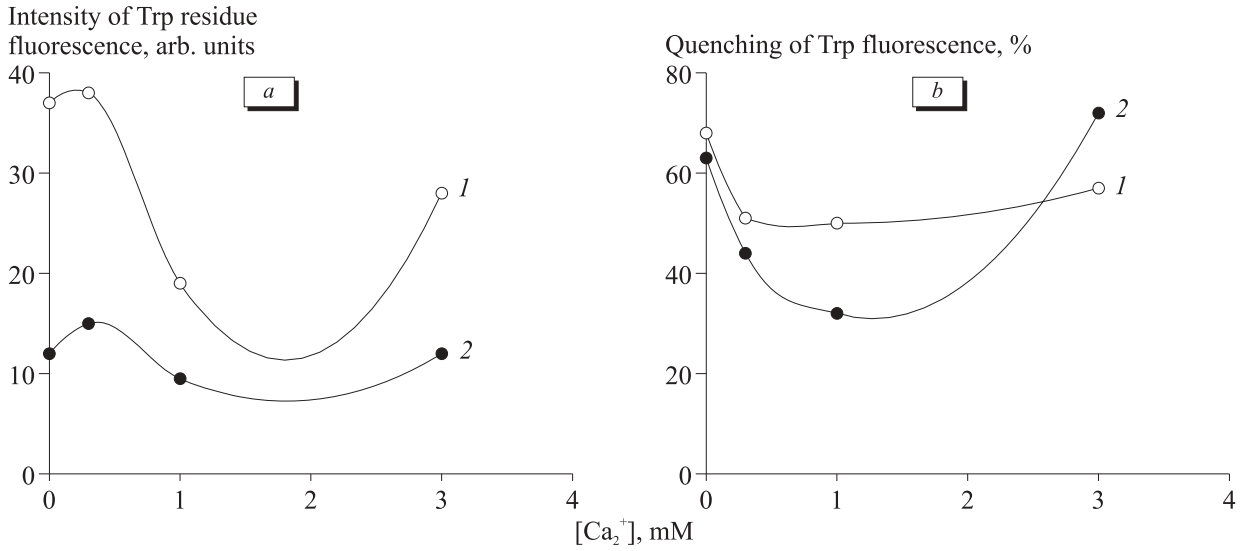

Fig. 1. Relationship between Ca^{2+} gradient and fluorescence of Trp residues in FSR fractions. *a*) pyrene effect on the relationship between Trp fluorescence of FSR heavy fraction and Ca^{2+} gradient. *1*) FSR; *2*) FSR with pyrene. *b*) relationship between Ca^{2+} gradient and pyrene quenching of Trp fluorescence. *1*) heavy fraction; *2*) light fraction.

Ca^{2+}. This can be explained by oligomerization of Ca^{2+}-ATPase proteins.

The effect of Ca^{2+} gradient on the lipid phase was evaluated by the fluorescence of two pyrene forms (monomer and eximer). Pyrene in low concentrations exists mainly in the form of monomers. They are located in the fatty acid residue area and migrate in all directions. The fluorescence (λ_{em}=390 nm) originates from phospholipid heads least saturated with fluorescence quencher (oxygen) [8]. At high concentrations pyrene, if the membrane has sufficiently large areas for dimer formation, forms eximers fluorescing at λ_{em}=480 nm. They are located near fatty acid residues, because their migration in the bilayer is impeded in all directions. The balance between pyrene eximer and monomer fractions depends on the general structure of the membrane: viscosity of lipid bilayer and presence of areas sufficient for the formation of large forms [8]. Fluorescence of the two pyrene forms reflects the characteristics of the entire bilayer. The relationships between the fluorescence of pyrene monomeric and eximeric forms and Ca^{2+} gradient concentration were different (Fig. 2, *a*, *b*). The maximum fluorescence of monomers was observed at 1 mM Ca^{2+}. For eximers, the dependence on the Ca^{2+} concentration gradient virtually did not change. This means that the most pronounced changes occurred in the surface areas of the bilayer (phospholipid head areas), while deep areas (fatty acid residues) did not change. Saturation of deep layers with oxygen seems to remain unchanged as well, because eximer fluorescence was not quenched in the entire range of studied values.

The greatest changes in the outer bilayer leaflet depending on the Ca^{2+} concentration gradient were demonstrated using merocyanine-540. It binds to loosely packed fatty acid residues in the outer bilayer leaflet or remains associated on the surface of compact bilayer. The intensity of its fluorescence depends on membrane potential and type of binding to the membrane. The location of merocyanine-540 fluorescence intensity peaks observed in our study for all three fractions at λ_{em}=585 nm [10] for the entire range of Ca^{2+} concentration gradient values indicates that merocyanine-540 incorporates into disordered and hence, liquid outer leaflet of the bilayer contacting with unsaturated fatty acid residues. Funnel-shaped curves of merocyanine-540 fluorescence intensity were plotted for all three FSR fractions with the maximum at 1 mM Ca^{2+} (Fig. 3). The relationship for the caffeine-insensitive fraction was most pronounced.

Hence, spectral analysis of the fluorescence of three fluorophores characterized changes in the structure of different sites of all compartments of the SR membrane. Emission of Trp residues [11] detected conformation changes in proteins incorporated into the membrane. The interaction between proteins and the nearest lipid environment was characterized by pyrene quenching of the fluorescence of Trp residues. Comparison of the emission of two pyrene forms [8] detected the characteristics of the lipid bilayer throughout its entire thickness. Fluorescence of merocyanine-540 [12] showed characteristics of the outer bilayer leaflet: compactness of the lipid packing and membrane potential. The detected extreme values at 1 mM Ca^{2+} concentration

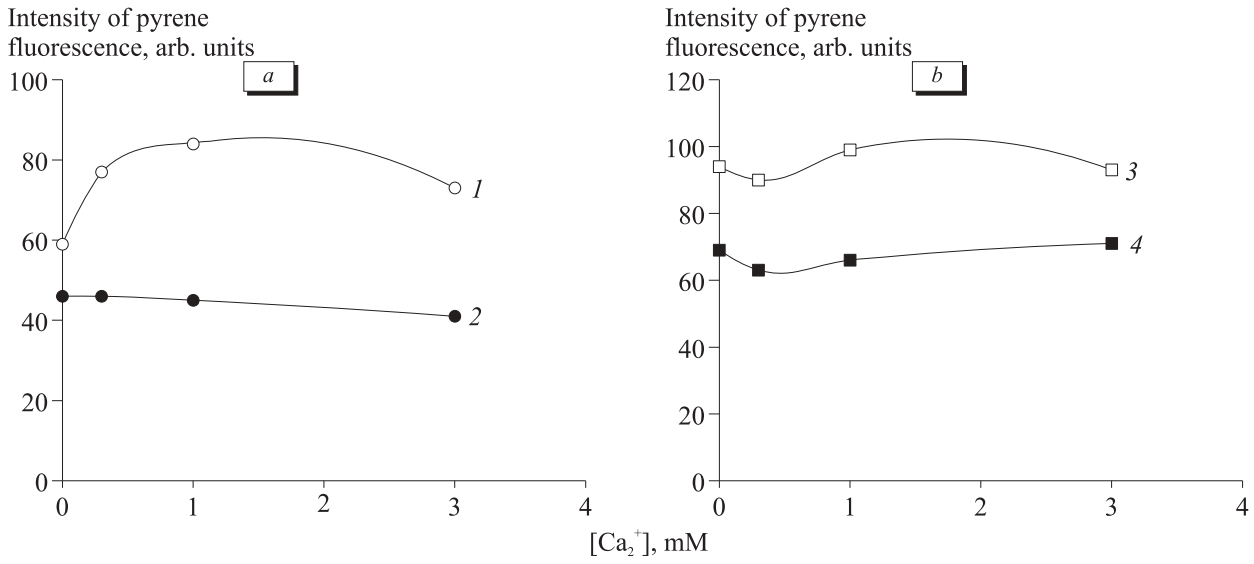

Fig. 2. Effect of Ca^{2+} gradient on the fluorescence of pyrene monomers and eximers in the FSR light fraction. *a*) caffeine sensitive, *b*) caffeine-insensitive fraction. *1-2*: monomers; *3-4*: eximers.

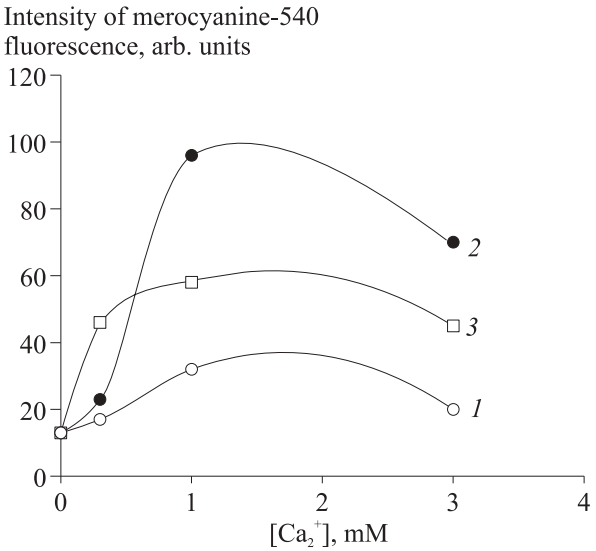

Fig. 3. The effect of Ca^{2+} gradient on merocyanine-540 fluorescence in FSR fractions. *1*) heavy; *2*) light caffeine-sensitive; *3*) light caffeine-insensitive fraction.

gradient not detected in pure lipid vesicles indicate that the transmembrane Ca^{2+} gradient is essential for the protein-lipid interactions, as a result of which the structural characteristics of SR membrane are modified. These changes were maximum at a gradient equal to 1 mM. Protein oligomerization can be responsible for the reduced availability of protein Trp residues for fluorescence quenchers (pyrene). The increase in merocyanine-540 fluorescence suggests that Ca^{2+} gradient affects surface charge. Hence, at the Ca^{2+} gradient corresponding to skeletal muscle cell status at rest, the proteins and lipids are redistributed in the membrane and the protein-lipid interactions and charge are modified. Presumably, the membrane structure is changed at the Ca^{2+} gradient of 1 mM and consequently, the mutual regulation of all components of Ca^{2+} depot (Ca^{2+} releasing channels, Ca^{2+} pump, Ca^{2+} binding luminal proteins and lipids) is modified, due to which the system is balanced and rapidly reacts to stimulation.

Evaluation of $[Ca^{2+}]_{SR}/[Ca^{2+}]_{cyt}$ effect for SR membranes (depot Ca^{2+}) is also important in pharmacological studies, because the gradient determines drug efficiency. For example, caffeine and quercetin modulate Ca^{2+} channel at certain $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{SR}$, when the target is maximally sensitive [13]. Moreover, the $[Ca^{2+}]_{SR}/[Ca^{2+}]_{cyt}$ determines the collective behavior of ryanodine receptor Ca^{2+} channels throughout Ca^{2+} release from the terminal cisterns during stimulation [14]. Opening of the Ca^{2+} channel can be electrochemically coupled with transmembrane $[Ca^{2+}]_{SR}/[Ca^{2+}]_{cyt}$. These are intricate interrelationships, because Ca^{2+} plays a dual role: as a penetrating cation and a ryanodine receptor regulator by the CIRC (calcium induced calcium release) mechanism [15].

Using this model in studies of pathological processes in muscle tissue and in their therapy, it is possible to create a transmembrane Ca^{2+} gradient, one of the key factors regulating function of the skeletal muscle SR Ca^{2+} depot, which helps to investigate the mechanisms of Ca^{2+} release and absorption, induced by drugs and endogenous regulators.

REFERENCES

1. N. A. Beard, M. M. Sakowska, A. F. Dulhunty, and D. R. Laver, *Biophys. J.*, **82**, No. 1, Pt. 1, 310-320 (2002).
2. D. M. Bers, *Circ. Res.*, **87**, No. 4, 275-281 (2000).
3. N. Ikemoto and T. Yamamoto, *Biochem. Biophys. Res. Commun.*, **279**, No. 3, 858-863 (2000).
4. M. Inui and S. Fleischer, *Methods in Enzymol.*, **157**, 490-505 (1988).
5. J. W. Lagerberg, J. Van Steveninck, and T. M. Dubbelman, *Cell. Mol. Life Sci.*, **53**, No. 3, 257-262 (1997).
6. E. H. Lee, G. Meissner, and D. H. Kim, *Biophys. J.*, **82**, No. 3, 1266-1277 (2002).
7. B. E. Murray and K. Ohlendieck, *FEBS Lett.*, **429**, No. 3, 317-322 (1998).
8. Y. Saiki and N. Ikemoto, *Biochem. Biophys. Res. Commun.*, **241**, No. 1, 181-186 (1997).
9. Y. Saiki and N. Ikemoto, *Biochemistry*, **38**, No. 10, 3112-3119 (1999).
10. N. L. Vekshin, *Energy Transfer in Macromolecules*, Bellingham-SPIE (1997), P. 104.
11. N. L. Vekshin, *Photonics of Biopolymers*, Berlin (2002), P. 229.
12. O. M. Vekshina, *Membr. Cell Biol.*, **14**, No. 4, 429-461 (2001).
13. O. M. Vekshina and N. L. Vekshin, *Mol. Biol.* (Moscow), **23**, No. 4, 1041-1050 (1989).
14. H. Yu and S. W. Hui, *Biochim. Biophys. Acta*, **1107**, No. 2, 245-254 (1992).
15. S. Q. Wang, L. S. Song, L. Xu, *et al.*, *Biophys. J.*, **83**, No. 1, 242-251 (2002).