LETTER

RyR1 Expression and the Cell Boundary Theorem

Eltit and colleagues (1) show that expression of RyR1 is associated with a number of changes in "dyspedic" skeletal myotubes, notably a doubling in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$). Physiologists have been aware for some time of a law, recently reformulated as the "cell boundary theorem" (2), which states that changes of resting $[Ca^{2+}]_c$ must be justified entirely by changes in the properties of the plasma membrane. In true resting state, an equality of fluxes must prevail (see Equation 1)

$$\text{influx}\,([Ca^{2+}]_e,\ [Ca^{2+}]_c) = \text{efflux}\,([Ca^{2+}]_e,\ [Ca^{2+}]_c) \quad \text{(Eq. 1)}$$

where influx and efflux are unidirectional fluxes across the plasma membrane. In turn these fluxes are determined by the concentrations of calcium, $[Ca^{2+}]_e$ and $[Ca^{2+}]_c$, on both sides, and the properties of the transport systems, pumps, carriers, and channels, present in the membrane.

In the study of reference the primary agent of change is the introduction of RyRs in the sarcoplasmic reticulum (SR) membrane, but these channels do not enter Equation 1, which defines the final value of $[Ca^{2+}]_c$. They must be causing the change in $[Ca^{2+}]_c$ indirectly.

It is now a rule that increase in "leak" from SR results in greater $[Ca^{2+}]_c$. Additional examples include malignant hyperthermia (MH) susceptibility RyR mutations (3), overexpression of RyR3 (4), and ablation of calsequestrin, which presumably releases RyRs from inhibition and causes MH-like leaks (5). Increases in leak thus promote net Ca^{2+} entry, which requires increase in influx, decrease in efflux, or both. The flux imbalance that modifies $[Ca^{2+}]_c$ must be transient, but the changes in flux may be permanent. Indeed, Eltit and colleagues (1) report a near doubling of resting influx.

The boundary theorem tells us more. The authors infer from their results that SERCA (sarco(endo)plasmic reticulum Ca^{2+}-ATPase) must be modified. However, according to the theorem a modified resting $[Ca^{2+}]_c$ only implies changes at the plasma membrane; changes in organellar membranes cannot be inferred from these observations.

A boundary theorem is applicable to organelles as well. For the resting SR it becomes as shown in Equation 2.

$$\text{uptake}\,([Ca^{2+}]_c,\ [Ca^{2+}]_{SR}) = \text{leak}\,([Ca^{2+}]_c,\ [Ca^{2+}]_{SR}) \quad \text{(Eq. 2)}$$

This implies that the presence of newly expressed RyRs (increasing leak) plus the observed 4-fold reduction of SERCA expression (decreasing uptake) ought to result in very severe reduction of $[Ca^{2+}]_{SR}$ if nothing else changed. The observed conservation of SR load therefore indicates that other determinants of uptake must have changed radically. The observed 2-fold increase in $[Ca^{2+}]_c$ could explain the steady load, but only if uptake depended on $[Ca^{2+}]_c$ in a highly non-linear manner.

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DOI 10.1074/jbc.L110.107300

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