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Review

Ryanodine receptors as leak channels

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ABSTRACT

Ryanodine receptors are Ca^{2+} release channels of internal stores. This review focuses on those situations and conditions that transform RyRs from a finely regulated ion channel to an unregulated Ca^{2+} leak channel and the pathological consequences of this alteration. In skeletal muscle, mutations in either $Ca_V1.1$ channel or RyR1 results in a leaky behavior of the latter. In heart cells, RyR2 functions normally as a Ca^{2+} leak channel during diastole within certain limits, the enhancement of this activity leads to arrhythmogenic situations that are tackled with different pharmacological strategies. In smooth muscle, RyRs are involved more in reducing excitability than in stimulating contraction so the leak activity of RyRs in the form of Ca^{2+} sparks, locally activates Ca^{2+}-dependent potassium channels to reduce excitability. In neurons the enhanced activity of RyRs is associated with the development of different neurodegenerative disorders such as Alzheimer and Huntington diseases. It appears then that the activity of RyRs as leak channels can have both physiological and pathological consequences depending on the cell type and the metabolic condition.

Contents

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1. Introduction

Ryanodine receptor (RyR) is a Ca^{2+} permeable non-selective cation channel that releases Ca^{2+} stored in the sarco-endoplasmic reticulum of excitable and non-excitable cells. This ion channel is a tetramer and each subunit contains approximately 5000 aa, so one single ion channel weights around 2 M Da. However, the part of RyRs forming the ion pore is located at the carboxy terminus and represents approximately 10% of the protein. In mammals, there are three different genes encoding for RyR1, RyR2 and RyR3 isoforms. RyR1 is abundantly expressed in skeletal muscle, particularly in fast twitch muscle; RyR2 is greatly expressed in heart cells and RyR3 was identified as an inducible protein in lung epithelial cells in response to TGF-β although it is constitutively expressed in brain, skeletal and smooth muscles and in non-excitable cells. Both RyR1 and RyR2 play a central role in the excitation contraction coupling in striated muscles. All three isoforms of RyRs are expressed in smooth muscle cells and yet, they play a minor role in excitation–contraction coupling in this type of muscle (Guerrero-Hernández et al., 2002; Iino et al., 1988). RyRs are also large ion pores, with an estimated single unitary current of 0.5 pA under quasi physiological conditions (Mejía-Alvarez et al., 1999), this is quite large, particularly for a Ca^{2+} permeable channel. It is clear then that the number and the activity of these large ion channels need to be under tight control. Moreover, it has been found that cells can express truncated RyRs comprised by the ion channel part only (Lee et al., 2002; Takeshima et al., 1993); although we do not know their role in cell physiology. A number of reviews on biochemical, physiological and genomic characteristics of RyRs have been published (Fill and Copello, 2002; Fleischer, 2008; Zalk et al., 2007). This review focuses on the physiological and non-physiological conditions that turn RyRs from tightly regulated channels into leaky pathways, which in turn alter cell physiology and associate with different pathological states both in muscle and non-muscle cells.

2. RyR as leak channels in skeletal muscle

2.1. RyR1s are not "intrinsically" leaky

The main role of RyR in striated muscle is to couple excitation with contraction by releasing Ca^{2+} from SR. However, skeletal and cardiac muscles express different subunits of either voltage-gated Ca^{2+} channels or ryanodine receptors ($Ca_V1.1$ and RyR1 in skeletal vs. $Ca_V1.2$ and RyR2 in cardiac). So, this leads to distinct excitation-contraction coupling (ECC) mechanisms. In skeletal muscle, a direct physical interaction between $Ca_V1.1$ and RyR1 rapidly transduces electrical depolarization of the plasma membrane in activation of RyR1 and the ensued SR Ca^{2+} release (known as voltage-gated Ca^{2+} release). In contrast, in cardiac muscle ECC depends on Ca^{2+} entry via $Ca_V1.2$ which in turn, binds to and activates RyR2, resulting in SR Ca^{2+} release (for reviews see Bannister (2007); Dulhunty (2006)).

Another interesting difference is that in contrast to cardiac muscle localized Ca^{2+} release events or sparks (which might be seen as manifestations of Ca^{2+} leak through RyRs) are rarely seen in mammalian skeletal muscle. In fact, in this tissue a leaky behavior of RyR1 is more commonly inferred from a reduction in steady-state levels of SR Ca^{2+}, which exclusively occurs in pathological situations. The absence of spontaneous opening of RyR1s may be due to an inhibitory action of $Ca_V1.1$ (Eltit et al., 2011; Zhou et al., 2006b). Additionally, it has been proposed that the C-terminal region of RyR1s makes them poorly sensitive to activation by luminal SR Ca^{2+} as opposed to RyR2s; (Kong et al., 2007).

2.2. Leak in RyR1 mutant channels

Mutations in the gene encoding RyR1 have been linked to various human diseases (for a recent update see Løseth et al. (2013)). Malignant hyperthermia susceptibility (MH) and central core disease (CCD) were the first in being associated with a mutated RyR1 gene, so these modifications are well characterized (Fujii et al., 1991; Quane et al., 1993; Zhang et al., 1993).

CCD is a congenital myopathy that leads to lower limb skeletal muscle weakness, atrophy, hypotonia, and skeletal deformities. The diagnosis is based on histopathological observation of "central cores", that are amorphous areas devoid of oxidative enzymatic activity and mitochondria. CCD patients often test positive for MH as well, which is a pharmacologically triggered and life threatening disease. The MH episodes are characterized by sudden rise in body temperature, hyper-metabolism, acidosis, tachycardia and skeletal muscle rigidity. In general, they are triggered by exposure to inhalant anesthetics and muscle relaxants, but can also be elicited by high temperature and stress. Dantrolene, which is thought to act by inhibiting the activity of RyR1s, represents currently the only antidote and is very effective in preventing the attacks (if applied intravenous promptly), when the initial symptoms appear (Betzenhauser and Marks, 2010; Lanner et al., 2010).

Functional studies of disease-linked mutations in RyR1 have resulted in models that attempt to explain the most conspicuous symptoms (muscle rigidity in MH and weakness in CCD)

(i) Mutations located near to the C-terminal that leave intact gating properties but impair Ca^{2+} conductance of RyR1 (Avila and Dirksen, 2001; Avila et al., 2003b; Zvaritch et al., 2007). The reduced Ca^{2+} conductance is thought to result only in CCD (CCD-only mutants), since it does not involve increased sensitivity to activation by either pharmacological compounds or $Ca_V1.1$. However, it causes decreased amplitude of Ca^{2+} transients from a normal SR Ca^{2+} content, likely contributing to muscle weakness in CCD. The corresponding functional phenotype, termed "EC uncoupling" (Avila et al., 2001), results also from mutations that promote a reduction in ECC units due to a drastic decline in RyR1 protein content; (Zhou et al., 2006a).

(ii) Mutations that produce an extremely leaky behavior of RyR1 and therefore decrease the SR Ca^{2+} content and increase cytosolic Ca^{2+} concentration ("decompensated leak"; (Avila and Dirksen, 2001; Tong et al., 1999)). These alterations in turn result in reduced amplitude of Ca^{2+} transients elicited by voltage activation of $Ca_V1.1$, which might contribute to muscle weakness in CCD. Individuals expressing these mutant proteins are also MH susceptible (i.e. MH+CCD mutants), probably due to an exacerbated response to MH triggering agents, which mobilizes Ca^{2+} excessively even from a partially depleted store (Dirksen and Avila, 2004).

(iii) Mutations that result in defective RyR1s that are moderately leaky and do not alter the steady-state distribution of Ca^{2+} across the SR lumen and cytoplasm ("compensated leak"; (Dirksen and Avila, 2004; Tong et al., 1999). These mutants do not alter the amplitude of voltage-gated Ca^{2+} release and thus should not produce muscle weakness, which conforms to its association with only MH (i.e. MH-only mutants).

Although these mechanisms have been accepted in general, the presence of contradictory experimental results and unsolved questions call for future work. An important question that remains open is: what is the primary defect responsible for increased Ca^{2+} fluxes in MH mutants? At least three fundamentally different defects have been proposed: (i) alterations in the sensitivity to