EXCITATION–CONTRACTION COUPLING IN SKELETAL MUSCLE: COMPARISONS WITH CARDIAC MUSCLE

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SUMMARY

1. The present review describes the mechanisms involved in controlling \( \text{Ca}^{2+} \) release from the sarcoplasmic reticulum (SR) of skeletal muscle, which ultimately regulates contraction.

2. Comparisons are made between cardiac and skeletal muscle with respect to: (i) the role of the dihydropyridine receptors (DHPR) as \( \text{Ca}^{2+} \) channels and voltage-sensors; (ii) the regulation of the ryanodine receptor (RyR)/\( \text{Ca}^{2+} \)-release channels in the SR; and (iii) the importance of \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release.

3. It is shown that the key differences of the skeletal muscle \( \text{Ca}^{2+} \)-release channel (RyR1), namely the increase in its stimulation by ATP and its inhibition by Mg\(^{2+} \), are critical for its direct regulation by the associated DHPR and, consequently, for the fast, accurate control of skeletal muscle contraction.

Key words: calcium release channels, dihydropyridine receptor, excitation–contraction coupling, muscle fatigue, ryanodine receptor, skeletal muscle, voltage-sensor.

OVERVIEW OF EXCITATION–CONTRACTION COUPLING IN CARDIAC AND SKELETAL MUSCLE

Skeletal muscle has a high performance role requiring very fast and yet highly accurate contraction. To achieve such performance characteristics, vertebrate skeletal muscle has evolved a unique control mechanism for regulating the \( [\text{Ca}^{2+}] \) in the cytoplasm, which, in turn, regulates force development by the contractile apparatus. To appreciate the unique features of this excitation–contraction (E–C) coupling mechanism in vertebrate skeletal muscle, it is worth first considering the comparable coupling mechanism in cardiac muscle. In a cardiac cell (see Fig. 1a), action potentials passing over the surface membrane activate voltage-sensitive (L-type) \( \text{Ca}^{2+} \) channels that rapidly open, allowing extracellular \( \text{Ca}^{2+} \) to flow into the cytoplasm. These voltage-sensitive \( \text{Ca}^{2+} \) channels are a type of dihydropyridine receptor (DHPR) and dihydropyridines, such as nifedipine, block channel opening. The \( \text{Ca}^{2+} \) entering the cell binds to and opens specialized \( \text{Ca}^{2+} \)-release channels (known as ryanodine receptors type 2; RyR2) in the nearby sarcoplasmic reticulum (SR) membrane and the \( \text{Ca}^{2+} \) flowing out of the SR activates other RyR2/\( \text{Ca}^{2+} \)-release channels, thereby reinforcing \( \text{Ca}^{2+} \) release. This is known as ‘\( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release’ (CICR) and, in the case of cardiac muscle, this term refers to activation of the release channels by \( \text{Ca}^{2+} \) coming both from outside the cell and from within the SR. There are only approximately one-tenth as many DHPR as \( \text{Ca}^{2+} \)-release channels, but that is all that is needed to activate all the release channels (Fig. 1a). The rise in cytoplasmic \( [\text{Ca}^{2+}] \) activates the contractile apparatus, producing force, and the \( \text{Ca}^{2+} \) is subsequently taken up into the SR and extruded from the cell by Na\(^{+}\)/\( \text{Ca}^{2+} \) exchange and \( \text{Ca}^{2+} \) pumping.

Excitation–contraction coupling in vertebrate skeletal muscle differs substantially from that in cardiac muscle but, in fact, is mediated by homologous proteins (Fig. 1b). In skeletal muscle, the action potentials pass along the surface membrane and into a network of invaginations known as the transverse tubular system (t system), which is far more extensive than in cardiac muscle. The depolarization is detected by DHPR molecules, also known as ‘voltage-sensors’\(^{2,3} \), which are a different L-type channel isoform than in cardiac muscle. Importantly, these DHPR only mediate a small, very slow influx of extracellular \( \text{Ca}^{2+} \) (Fig. 2b)\(^{5,6} \) and, instead, in some way directly activate the \( \text{Ca}^{2+} \)-release channels in the adjacent SR. Adult vertebrate skeletal muscle can contract vigorously in the absence of extracellular \( \text{Ca}^{2+} \), showing unequivocally that \( \text{Ca}^{2+} \) inflow is not needed for initiating \( \text{Ca}^{2+} \) release.\(^{3,6,7} \) (Interestingly, invertebrate skeletal muscle and neonatal vertebrate skeletal muscle are more like cardiac muscle, requiring inflow of extracellular \( \text{Ca}^{2+} \) for full contraction.)\(^{3,5} \) It is also important to note that \( \text{Ca}^{2+} \) release from the SR in skeletal muscle remains under close control by the voltage sensors at all times and stops rapidly if the voltage sensors are no longer activated, showing that the raised \( [\text{Ca}^{2+}] \) in the cytoplasm is not sufficient by itself to maintain \( \text{Ca}^{2+} \) release.

To enable direct coupling between the DHPR and \( \text{Ca}^{2+} \)-release channels in skeletal muscle, there are a number of key modifications in the coupling proteins and their arrangement compared with that in cardiac muscle. First, the relative density of the DHPR in skeletal muscle is increased approximately five- to 10-fold,\(^{9,10} \) such that there is one DHPR complex (actually a ‘tetrad’ of four molecules; see
E-C coupling in skeletal vs cardiac muscle

Later) apposing every second Ca\(^{2+}\)-release channel \(1^1\) (see Fig. 1b). This increased density can be observed in binding and anatomical studies and is also evident from the larger amount of ‘asymmetric charge movement’ in skeletal muscle \(3\) compared with cardiac muscle \(4\) (compare Fig. 2a,b; see Lamb \(9\)). This electrical signal is generated by the voltage-induced activation of the DHPR and its magnitude is proportional to the number of DHPR present in the membrane. In skeletal muscle, only a small fraction of these DHPR actually open as Ca\(^{2+}\) channels and do so slowly, resulting in a much smaller, slower-activating inward Ca\(^{2+}\) current than in cardiac muscle; see Fig. 2.) Second, the intracellular projections of the skeletal DHPR are modified for direct interaction with the release channels \(12\) (see later) and the ability of the DHPR to allow influx of extracellular Ca\(^{2+}\) is reduced (Fig. 2b). Third, the Ca\(^{2+}\)-release channel in mammalian skeletal muscle is also a different isoform (RyR1), able to interact with the DHPR, \(^{13}\) and with crucial changes to its regulation by cytoplasmic Ca\(^{2+}\), Mg\(^{2+}\) and ATP (see later). It is also worth noting that skeletal muscle fibres are relatively closed compartments with regard to Ca\(^{2+}\) movements. During activity, very little Ca\(^{2+}\) flows into the fibre due to the reduced Ca\(^{2+}\) channel function of the DHPR and virtually all the Ca\(^{2+}\) released into the cytoplasm is eventually pumped back into the SR, with very little being extruded from the fibre by either Na\(^+\)/Ca\(^{2+}\) exchange or Ca\(^{2+}\) pumping. Thus, the reduced calcium channel function of the skeletal DHPR increases energetic efficiency because it prevents the need for subsequent Ca\(^{2+}\) extrusion. Furthermore, it may be important in avoiding high local increases in cytoplasmic [Ca\(^{2+}\)] that may, inappropriately, affect the direct regulation of the Ca\(^{2+}\)-release channels by the DHPR.

TECHNIQUES FOR INVESTIGATING Ca\(^{2+}\) RELEASE IN SKELETAL MUSCLE

Many different techniques have been used to gain information about the regulation of Ca\(^{2+}\) release in skeletal muscle, each having advantages and drawbacks. Studies using Ca\(^{2+}\) indicators inside intact fibres and voltage-clamped cut fibres allow accurate control of voltage sensor activation, \(^{2,3,6}\) but the intracellular environment cannot be rapidly manipulated. Studies with SR vesicles and isolated RyR in artificial bilayers allow systematic examination of the effects of cytoplasmic and SR luminal factors on the properties of the RyR, \(^{14}\) but the normal voltage sensor control of the RyR is absent (or, in the case of SR ‘triad’ preparations, present but possibly modified). Another technique is to use mechanically skinned muscle fibres (Fig. 3a), where the surface membrane is removed to allow ready manipulation of the intracellular environment while still retaining normal voltage sensor control of Ca\(^{2+}\) release. \(^{15,16}\) The principal drawbacks with this technique are that the t system potential is not accurately controlled and the rate of depolarization and repolarization is limited by the time taken for solutions to diffuse into and out of the skinned fibre (approximately 0.3–1 s). However, one additional advantage of the mechanically skinned fibre technique is
that if the fibres are skinned under paraffin oil the SR of the skinned fibre is initially loaded with Ca\(^{2+}\) at the endogenous level and it can be kept at approximately that level.\(^\text{15}\) This is particularly important because the level of Ca\(^{2+}\) loading in the SR appears to have a great influence on the properties of the release channels (see Sitsapesan and Williams\(^\text{17}\)).

**FINDINGS FROM SKINNED FIBRES**

The depolarization-induced responses elicited in skinned fibres (e.g. Fig. 3b) are analogous to potassium contractures in intact fibres.\(^\text{7}\) It is significant that many such responses can be elicited successively over a period more than 30 min.\(^\text{15,18}\) Because the normal cytoplasm has been entirely washed out and replaced with a minimal ‘physiological’ solution (high [K\(^+\)], 8 mmol/L ATP, 10 mmol/L creatine phosphate, 1 mmol/L free Mg\(^{2+}\), 0.1 \(\mu\)mol/L Ca\(^{2+}\), pH 7.1), it is apparent that the coupling mechanism is extremely robust and has few essential myoplasmic requirements for function. This further supports the proposal that the DHPR/voltage sensors are physically coupled to the RyR, either directly or via some other tightly associated molecule. There does not seem to be any diffusible ‘second messenger’ required to mediate the coupling. Additional experiments argue specifically against inositol 1,4,5-trisphosphate (IP\(_3\)) having such a role (see Posterino and Lamb\(^\text{19}\)). Similarly, Ca\(^{2+}\) itself does not act as such a messenger. Although entry of extracellular Ca\(^{2+}\) is clearly not necessary for the coupling, it could be argued that depolarization may release Ca\(^{2+}\) bound on the cytoplasmic face of the t system, which would then activate the Ca\(^{2+}\)-release channels. However, this seems not to be the case because: (i) the t system depolarization can still initiate Ca\(^{2+}\) release in cut and skinned fibres, despite the presence in the cytoplasm of high

![Fig. 2](image-url)  
**Fig. 2**  
Asymmetric charge movement and Ca\(^{2+}\) currents in (a) cardiac and (b) skeletal muscle cells under voltage clamp. (a) In this neonatal rat cardiac cell, a (20 ms) voltage step to +20 mV rapidly activates an inward Ca\(^{2+}\) current (downward deflection) through the voltage-sensitive Ca\(^{2+}\) channels (dihydropyridine receptors; DHPR) in the surface membrane. The inward Ca\(^{2+}\) current is preceded by a small outward current (‘asymmetric charge movement’; upward deflection) that is generated by the initial conformational change in the DHPR as they respond to the depolarization. (b) When a rat skeletal muscle (extensor digitorum longus) fibre is subjected to a similar stimulus (30 ms step to +20 mV), the activation of the numerous DHPR/voltage sensors generates a large amount of asymmetric charge movement, but only a small proportion of these DHPR actually open as Ca\(^{2+}\) channels and do so relatively slowly, resulting in a small, slowly activating inward Ca\(^{2+}\) current. Rat cells are in 2 mmol/L extracellular Ca\(^{2+}\) in both (a) and (b). (Modified from Lamb & Walsh\(^\text{4}\) and Field \(et\ al.\)^\text{5}).

![Fig. 3](image-url)  
**Fig. 3**  
(a) Schematic diagram of a mechanically skinned skeletal muscle fibre. When the surface membrane is peeled away by microdissection, the t system seals off and, if the skinned segment is bathed in a solution mimicking the normal cytoplasm (i.e. high [K\(^+\)], some Na\(^+\), ATP, 1 mmol/L free Mg\(^{2+}\) etc.), the Na\(^+\)–K\(^+\) pumps in the t system membrane establish a high [Na\(^+\)]–low [K\(^+\)] inside the t system, thus polarizing it like in an intact fibre. The t system can be rapidly depolarized (< 1 s) by transferring the skinned fibre segment to a zero [K\(^+\)]–high [Na\(^+\)] solution (Na\(^+\) substitution), which triggers Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) via the normal voltage sensor-controlled pathway. The resulting contraction can be measured with a force transducer. DHPR, dihydropyridine receptor; RyR1, ryanodine receptor type 1. (b) Depolarization-induced force responses elicited by Na\(^+\) substitution in a skinned extensor digitorum longus fibre of the rat. Such responses can be elicited repeatedly, provided that the t system is repolarized in the high [K\(^+\)] for 30–60 s in between, before the fibre eventually becomes unresponsive. The 12th and 20th responses are shown. Time scale, 2 s during depolarizations (bars), 30 s elsewhere.
concentrations of the fast Ca\textsuperscript{2+}-chelator BAPTA\textsuperscript{2,20} and (ii) Ca\textsuperscript{2+} does not readily initiate fast Ca\textsuperscript{2+} release from the SR in skeletal muscle at physiological levels of cytoplasmic [Mg\textsuperscript{2+}] and SR Ca\textsuperscript{2+} loading, at least in the case of mammalian muscle.\textsuperscript{21-24} This latter point is particularly important, because it means not only that Ca\textsuperscript{2+} would be relatively ineffective as a messenger between the t system and the release channels, but also that (in the absence of voltage sensor activation) skeletal Ca\textsuperscript{2+}-release channels are not potently activated by Ca\textsuperscript{2+} released through neighbouring channels. This is in accord with the observations that ‘Ca\textsuperscript{2+} sparks’, which are thought to be due to localized Ca\textsuperscript{2+} activation of neighbouring release channels, are prominent in cardiac muscle but are entirely absent in mammalian skeletal muscle and are comparatively uncommon in amphibian muscle in the absence of voltage sensor activation.\textsuperscript{25} Furthermore, it fits with the finding that caffeine, which triggers Ca\textsuperscript{2+} release by augmenting Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release, is actually poorly effective in mammalian skeletal muscle, eliciting only very small slow-force responses in intact polarized fibres, even at 10–15 mmol/L.\textsuperscript{26,27}

**ACTIVATION OF RyR BY Ca\textsuperscript{2+} AND ATP AND INHIBITION BY Mg\textsuperscript{2+}**

The relative inability of Ca\textsuperscript{2+} to activate the Ca\textsuperscript{2+}-release channels in mammalian skeletal muscle is due primarily to the presence of Mg\textsuperscript{2+} in the cytoplasm. The Mg\textsuperscript{2+} exerts a powerful inhibitory effect on the release channels in skeletal muscle under resting conditions and, if the free [Mg\textsuperscript{2+}] is lowered well below its physiological level of 1 mmol/L (see Lamb and Stephenson\textsuperscript{28}) to approximately 0.05 mmol/L, the Ca\textsuperscript{2+}-release channels spontaneously open and all the Ca\textsuperscript{2+} is lost from the SR.\textsuperscript{20,23} (e.g. Fig. 4). Lowering the [Mg\textsuperscript{2+}] induces Ca\textsuperscript{2+} release even in the virtual absence of Ca\textsuperscript{2+} (< 10 nmol/L), because the skeletal Ca\textsuperscript{2+}-release channels are also strongly stimulated by the presence of ATP in the cytoplasm.\textsuperscript{23}

These findings in skinned fibres, where voltage sensor control of Ca\textsuperscript{2+}-release channels is still intact and functional, are in good agreement with the properties of the Ca\textsuperscript{2+}-release channels quantitatively characterized in studies with SR vesicles and isolated channels from mammalian muscle.\textsuperscript{14,22,29-31} These latter studies show that the cardiac Ca\textsuperscript{2+}-release channel is stimulated by Ca\textsuperscript{2+} binding at an activation site with a dissociation constant (K\textsubscript{a}) of approximately 1 \(\mu\text{mol/L}\) and is inhibited by Ca\textsuperscript{2+} binding at a very low affinity site (K\textsubscript{i} > 1 mmol/L), with ATP binding having only a mild stimulatory effect (Fig. 5a). This gives a bell-shaped Ca\textsuperscript{2+} dependence for channel activation in RyR2 (upper curve in Fig. 6a, no Mg\textsuperscript{2+}). The regulation of the mammalian skeletal Ca\textsuperscript{2+}-release channel (RyR1) by Ca\textsuperscript{2+} and ATP differs in two major ways: (i) ATP binding to a low-affinity site (K\textsubscript{s} approximately 1 mmol/L) stimulates channel opening even in the absence of cytoplasmic Ca\textsuperscript{2+}; and (ii) the affinity of the inhibitory site for Ca\textsuperscript{2+} is appreciably higher than in RyR2 (K\textsubscript{i} approximately 0.1 mmol/L at physiological ionic strength; Fig. 5b). This means that, in the presence of ATP, the bell-shaped curve for Ca\textsuperscript{2+} dependence of RyR1 (Fig. 6b) differs from that of RyR2 (Fig. 6a) in that it shows substantial activity at very low [Ca\textsuperscript{2+}] (< 10 nmol/L) and marked inhibition at 0.1–1 mmol/L Ca\textsuperscript{2+}. Thus, the bell-shaped curve for RyR1 (Fig. 6b, upper curve, in absence of Mg\textsuperscript{2+}) has its activation arm (left side) shifted upwards and its inhibition arm (right side) shifted to 10-fold lower [Ca\textsuperscript{2+}] compared with that for RyR2 (Fig. 6a, upper curve).

Now it is critically important to consider the effects of cytoplasmic Mg\textsuperscript{2+} on the RyR. In RyR1 and RyR2, Mg\textsuperscript{2+} competes with Ca\textsuperscript{2+} at both the activation and inhibition sites. The affinity of the activation site for Mg\textsuperscript{2+} is approximately 40–1000-fold lower than for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} binding does not activate the channel.\textsuperscript{22,23} Thus, the presence of 1 mmol/L Mg\textsuperscript{2+} simply shifts the Ca\textsuperscript{2+} dependency of activation to higher [Ca\textsuperscript{2+}] in both RyR1 and RyR2. In contrast, the affinity of the inhibitory site is virtually identical for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} and the binding of Mg\textsuperscript{2+} is just as effective at inducing inhibition as is the binding of Ca\textsuperscript{2+}.\textsuperscript{22,23} Thus, this non-specific site on both RyR1 and RyR2 should really be regarded as a ‘Mg\textsuperscript{2+}-inhibition site’ or a ‘Ca\textsuperscript{2+}/Mg\textsuperscript{2+} inhibition site’ because, with 1 mmol/L Mg\textsuperscript{2+} in the cytoplasm, it will be occupied by Mg\textsuperscript{2+} more than by Ca\textsuperscript{2+}, as the cytoplasmic [Ca\textsuperscript{2+}] is unlikely to reach millimolar levels, even locally, during peak Ca\textsuperscript{2+} release. This Ca\textsuperscript{2+}/Mg\textsuperscript{2+} inhibition is seemingly not of great importance in cardiac muscle, because the affinity of the site is so low (K\textsubscript{i} > 1 mmol/L). Consequently, the net effect of the presence of Mg\textsuperscript{2+} at 1 mmol/L\textsuperscript{23} on the Ca\textsuperscript{2+} dependent activity of RyR2 is a shift in the activation arm to higher [Ca\textsuperscript{2+}] and a small reduction in peak activation possible (i.e. to the same level as seen with 1 mmol/L Ca\textsuperscript{2+} in the absence of Mg\textsuperscript{2+}; Fig. 6a).

Thus, in a cardiac cell, a rise in myoplasmic [Ca\textsuperscript{2+}] to, say, 1 \(\mu\text{mol/L}\) could be expected to activate some release channels partially and the resulting rise in [Ca\textsuperscript{2+}] will potently reinforce this, opening nearby channels near maximally. In contrast, the skeletal Ca\textsuperscript{2+} release channel (RyR1) will be very greatly inhibited by the presence of 1 mmol/L Mg\textsuperscript{2+} due to Mg\textsuperscript{2+} binding to the Ca\textsuperscript{2+}/Mg\textsuperscript{2+} inhibitory site and, no matter how high the local [Ca\textsuperscript{2+}] rises, the RyR1 channels will not be able to be more than partially activated (i.e. to no more than the level seen with 1 mmol/L Ca\textsuperscript{2+} in the absence of Mg\textsuperscript{2+}; Fig. 6b).

This poor ability of Ca\textsuperscript{2+} to activate the mammalian skeletal Ca\textsuperscript{2+}-release channels under physiological conditions is not widely appreciated. This is probably because most studies characterizing the

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**Fig. 4** Intracellular Mg\textsuperscript{2+} exerts a powerful and important inhibitory effect on the Ca\textsuperscript{2+}-release channels in skeletal muscle. In a resting skinned muscle fibre, Ca\textsuperscript{2+} remains in the sarcoplasmic reticulum (SR) provided that the free [Mg\textsuperscript{2+}] is at its normal physiological level of approximately 1 mmol/L. If the intracellular [Mg\textsuperscript{2+}] is lowered considerably (e.g. to 0.05 mmol/L), the Ca\textsuperscript{2+}-release channels spontaneously open and all the Ca\textsuperscript{2+} is rapidly lost from the SR. Clearly: (i) Mg\textsuperscript{2+} is required to keep the release channels closed at rest; and (ii) the fact that t system depolarization can elicit Ca\textsuperscript{2+} release means that voltage sensor activation must, in some way, overcome or bypass the inhibitory effect of Mg\textsuperscript{2+} on the release channels. Depol., depolarization. (Reproduced from Lamb and Stephenson.\textsuperscript{23})
Fig. 5 Comparison of the regulation of the ryanodine receptor (RyR)/Ca\(^{2+}\)-release channels in (a) cardiac and (b) skeletal muscle. (a) The cardiac Ca\(^{2+}\) release channel (RyR2) is activated by Ca\(^{2+}\) binding to a cytoplasmic site with a dissociation constant (K\(_a\)) of approximately 1 \(\mu\)mol/L. It also can be inhibited by Ca\(^{2+}\) (or Mg\(^{2+}\)) binding to a second (non-specific) type of site, but because this site has such a very low affinity (K\(_i\) > 1 mmol/L) it is probably of little effect. Thus, rises in cytoplasmic Ca\(^{2+}\) potentiate activate RyR2. DHPR, dihydropyridine receptor; SR, sarcoplasmic reticulum. (b) In contrast, the skeletal Ca\(^{2+}\) release channel (RyR1) has a very similar Ca\(^{2+}\) activation site, but the non-specific inhibitory site has a much lower affinity than in RyR2 (K\(_i\) approximately 0.1 mmol/L) and would usually be occupied by Mg\(^{2+}\) normally present in the cytoplasm (1 mmol/L). In addition, ATP binding exerts a stronger stimulatory effect on RyR1 than on RyR2, but because RyR1 is normally under strong resting inhibition by Mg\(^{2+}\), it is not readily activated by either cytoplasmic Ca\(^{2+}\) or ATP. In order to potently activate RyR1, the voltage sensor/DHPR in the t system must overcome or bypass the inhibitory effect of Mg\(^{2+}\).

properties of the release channels understandably use conditions that facilitate activation, in particular heavily loading the SR with Ca\(^{2+}\) or reducing the inhibitory effect of Mg\(^{2+}\) by: (i) having little or no free Mg\(^{2+}\); or (ii) using high ionic strength or \([Cl^-]\), which greatly reduces the affinity of Mg\(^{2+}\) (and Ca\(^{2+}\)) for the inhibitory site.\(^{34}\)

**VOLTAGE SENSOR ACTIVATION OF Ca\(^{2+}\) RELEASE**

Even though the mammalian skeletal Ca\(^{2+}\)-release channels can be only weakly activated by cytoplasmic Ca\(^{2+}\) at physiological [Mg\(^{2+}\)], it is clear that the voltage sensor/DHPR can potently activate the channels under such circumstances in intact, cut and skinned fibres (e.g. Figs 3b,4). Thus, the voltage sensors must be able to overcome or bypass the strong inhibitory effect of Mg\(^{2+}\) on RyR1. Noting that the ability of the voltage sensors to trigger Ca\(^{2+}\) release is greatly inhibited in the presence of 10 mmol/L Mg\(^{2+}\) in skinned fibres from amphibian\(^{23}\) and mammalian skeletal muscle,\(^{26}\) it was proposed that voltage sensor activation in some way lowers the affinity of the inhibitory site on RyR1 for Mg\(^{2+}\) (and presumably Ca\(^{2+}\) too) by 10–20-fold. Such a mechanism seems even more plausible when it is noted that the affinity of the RyR1 inhibitory site for Mg\(^{2+}\) (and Ca\(^{2+}\)) can be lowered to a similar extent by a number of other treatments, such as oxidation, phosphorylation, increased ionic strength or application of the sponge extract bastadin.\(^{30,34–36}\) Such an effect of voltage sensor activation would alter the Ca\(^{2+}\)-dependent properties of RyR1, as shown in Fig. 7. The inhibition arm would be shifted to higher [Ca\(^{2+}\)]/[Mg\(^{2+}\)] and the strong inhibitory effect of 1 mmol/L Mg\(^{2+}\) exerted via the Ca\(^{2+}\)/Mg\(^{2+}\) inhibitory site would be removed, with the consequence that the channel would be open near maximally at intermediate [Ca\(^{2+}\)]. Importantly, because cytoplasmic ATP can stimulate channel opening even when there is no Ca\(^{2+}\) on the activation site, this voltage sensor-induced removal of the resting Mg\(^{2+}\) inhibition would result in the release channels opening appreciably at the normal resting [Ca\(^{2+}\)] (i.e. approximately 0.1 \(\mu\)mol/L; Fig. 7) and the released Ca\(^{2+}\) would then greatly reinforce channel opening now that it was no longer constrained by the inhibitory effect of Mg\(^{2+}\) (Fig. 7). Note that this is not a case of simple CICR because the Ca\(^{2+}\) is only able to potently activate the release channel while the voltage sensor is negating the inhibitory effect of Mg\(^{2+}\).

Thus, voltage sensor activation would induce full opening of RyR1 if it simply lowered the affinity of the inhibitory site for Mg\(^{2+}\) (and Ca\(^{2+}\)). Furthermore, when the voltage sensors were deactivated (by t system repolarization), the normal affinity of the site would be restored and Mg\(^{2+}\) would rebind and, once again, exert its strong inhibitory effect, thereby greatly reducing the opening of the release channels, even in the presence of a high local [Ca\(^{2+}\)] (Fig. 7). (Complete cessation of Ca\(^{2+}\) release may also be aided by a process in which individual RyR rapidly inactivate after activating.\(^{37,38}\) It is possible that voltage sensor activation also reduces the inhibitory effect of Mg\(^{2+}\) on the Ca\(^{2+}\)-activation site by increasing the relative affinity of that site for Ca\(^{2+}\) compared with Mg\(^{2+}\); this would marginally increase the initial rate of channel activation. However,
This possible additional action is actually not necessary, because the stimulatory effect of ATP would ensure initial channel activation in any case. Also, it is important to note that removing the inhibitory effect of Mg\(^{2+}\) at the Ca\(^{2+}\)-activation site would not be sufficient by itself to give full channel activation, because this would still be limited by inhibition exerted through the Ca\(^{2+}\)/Mg\(^{2+}\) inhibitory site. I emphasize that it has not actually been shown that the voltage sensor activation could not fully overcome this inhibition if it only reduces the affinity of the inhibitory site 10–20-fold (Fig. 7). The inhibitory effect of raised [Mg\(^{2+}\)] is likely to rise high enough to cause appreciable inhibition (e.g. > 0.5 mmol/L Ca\(^{2+}\)). Deactivation of the voltage sensors would restore the inhibitory effects of Mg\(^{2+}\) and, hence, dramatically reduce Ca\(^{2+}\) release, irrespective of the local activating [Ca\(^{2+}\)].
also seen with depolarization-induced Ca\(^{2+}\) release in SR triads,\(^{29}\) where raising either the [Mg\(^{2+}\)] alone or the total [Mg\(^{2+}\) + Ca\(^{2+}\)], from 0.7 to 1.5 mmol/L, caused complete loss of fast Ca\(^{2+}\) release. Because there was 0.3 mmol/L Ca\(^{2+}\) present with the 1.2 mmol/L Mg\(^{2+}\) in the latter case, this result can only be explained as being due to increased occupancy of the Ca\(^{2+}/Mg\(^{2+}\) inhibition site and not to competition of Mg\(^{2+}\) with Ca\(^{2+}\) for the activation site. In addition, it has been shown that depolarization-induced Ca\(^{2+}\) release in skinned fibres is also strongly inhibited if the [ATP] is reduced to only 0.5 mmol/L,\(^{41}\) consistent with ATP having to be bound to RyR1 for its normal activation. It is possible that, in a severely fatigued fibre, the [ATP] locally near the Ca\(^{2+}\)-release channels is much lower than in the bulk solution due to the absence of creatine phosphate resynthesis of ATP and the probable high local use of ATP by the SR Ca\(^{2+}\)-pumps surrounding the Ca\(^{2+}\)-release channels. Thus, Ca\(^{2+}\) release in fatigued fibres may be low because of both reduced ATP stimulation and increased Mg\(^{2+}\) inhibition of the Ca\(^{2+}\) release channels.\(^{41,42}\)

It also seems that an alteration in Mg\(^{2+}\) inhibition underlies at least some forms of MH. Malignant hyperthermia is an inherited muscle disease in which stress or volatile anaesthetics, such as halothane, can induce irreversible contractures in the skeletal muscle of susceptible humans and pigs. In pigs and some humans, MH is caused by a particular mutation in RyR1 (Arg\(^{615}\) to Cys). We have recently shown in experiments with isolated RyR that this mutation causes a reduction in the affinity of the Ca\(^{2+}/Mg\(^{2+}\) inhibitory site by approximately three-fold (K\(_i\) approximately 0.1 to 0.3 mmol/L, at normal ionic strength).\(^{34}\) Thus, this accounts well for the development of MH, because the mutated RyR1 is not as potently inhibited by the 1 mmol/L Mg\(^{2+}\) present in the cytoplasm and, consequently, any stimulus that in any way increases resting [Ca\(^{2+}\)] (e.g. halothane, caffeine, stress) could be expected to much more easily trigger a self-potentiating episode of CICR in an individual with the mutated RyR1 than in a normal individual (Fig. 6b).

**INTERACTION OF THE DHPR AND RyR**

Finally, to appreciate further how the DHPR may actually physically control the RyR1/Ca\(^{2+}\)-release channels in skeletal muscle, it is necessary to consider in more detail the arrangement of proteins at the triad junction, where the t system abuts the SR. The Ca\(^{2+}\)-release channel projects out from the SR towards the t system, with the large cytoplasmic ‘foot’ having a square appearance when viewed from the t system (Fig. 8). These channels are packed together in long crystal-like arrays all the way along the junctional face of the SR. Each RyR1/Ca\(^{2+}\)-release channel is composed of four identical isomers that, together, normally function to give all-or-none openings and closures. Each monomer is tightly associated with a 12 kDa FK506-binding protein (FKBP12),\(^{43}\) with one residing on each of the four outside faces of the cytoplasmic foot structure.\(^{44}\) The RyR are also tightly associated with calsequestrin, which is the principal Ca\(^{2+}\)-binding protein in the SR, and this may be how the amount of Ca\(^{2+}\) in the SR can affect Ca\(^{2+}\) release (Sitsapesan and Williams\(^{37}\)). Furthermore, the RyR interacts with a 90 kDa protein named triadin, but it is unclear whether triadin links the RyR to calsequestrin or to the DHPR (Franzini-Armstrong and Jorgensen\(^{11}\)). The low-affinity Ca\(^{2+}/Mg\(^{2+}\) inhibitory binding site on RyR1 seems likely to be formed by the diffuse negative charge associated with a sequence of more than 30 acidic nucleotides at residues 1870–1910 (Laver et al.\(^{35}\)).

It appears that every alternate Ca\(^{2+}\)-release channel is apposed by a group of four DHPR arranged in a diamond shape (a ‘tetrad’);\(^{11}\) this alternating arrangement may result from it not being physically

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**Fig. 8** Putative arrangement of triad proteins in skeletal muscle. Each dihydropyridine receptor (DHPR)/voltage sensor consists of four homologous ‘repeats’, and four such DHPR are clustered together in a diamond-shaped arrangement (a ‘tetrad’) apposing four ryanodine receptor (RyR) type 1 monomers that, together, function as a single Ca\(^{2+}\)-release channel. The intracellular loop between repeats II and III of each DHPR is thought to physically activate in some way the adjacent RyR monomers. A 12 kDa FK 506-binding protein (FKBP 12) is tightly associated with each RyR1 monomer and may regulate interactions both within and between adjacent RyR. Calsequestrin is the main Ca\(^{2+}\)-binding protein in the sarcoplasmic reticulum (SR) and possibly regulates the RyR. Triadin is another protein tightly associated with the RyR and has been suggested to mediate interactions with both the DHPR and calsequestrin.
possible to fit a ‘tetrad’ of DHPR opposite every release channel in the tightly packed array. Each DHPR is itself composed of four homologous ‘repeats’ and the intracellular loop between repeats II and III appears to play a vital part in the coupling with the RyR, as chimeric proteins composed of the cardiac DHPR with the skeletal DHPR II–III loop can mediate skeletal E-C coupling, whereas the cardiac DHPR themselves cannot. It has also been shown that a particular peptide segment of the II–III loop can activate RyR1 in skeletal SR vesicles, although it is not yet clear whether this is the basis of normal E-C coupling. Experiments are in progress to see whether these II–III loop peptides can trigger Ca\(^{2+}\) release in skinned fibres and how this relates to the normal coupling process.

Interestingly, FKBP12 seems to play an important part in the coupling process. The FKBP12 apparently enables the four RyR monomers to open and close in tandem, as they gate separately if FKBP12 is absent or dissociated by application of FK506. Furthermore, FKBP12 seems to facilitate the simultaneous gating of adjacent Ca\(^{2+}\)-release channels. This phenomenon may explain how every second Ca\(^{2+}\)-release channel, although not directly opposed by DHPR (Fig. 1), can still be activated appropriately. A further indication that FKBP12 is vital for normal coupling is that when skinned skeletal muscle fibres are treated with FK506 to dissociate FKBP12, depolarization-induced responses are greatly reduced or lost. Consequently, it is known that the native extract bastadin, which is known to lower the affinity of the Ca\(^{2+}\)/Mg\(^{2+}\) site on RyR1 by approximately 10-fold, appears to mediate its effects through the FKBP12 protein, and it seems quite plausible that communication between the DHPR and RyR1 is mediated in some way by FKBP12. A recent study in which FKBP12 expression was knocked out in mice found that mice died soon after birth of heart failure, but that those few surviving for a month or more had no gross changes in skeletal muscle function. Nevertheless, that study does not rule out an important role of FKBP12 in normal E-C coupling because: (i) FKBP12.6, a more sparsely expressed variant of FKBP12 that can replace FKBP12 on RyR1, was not also knocked out; and (ii) contraction in neonatal skeletal muscle, unlike adult muscle, appears to depend on extracellular Ca\(^{2+}\) influx and also on the activity of RyR3, a third type of RyR not appreciably present in adult fast-twitch muscle. Thus, overall, current evidence suggests that FKBP12 plays a vital role in the archetypal E-C coupling seen in adult mammalian skeletal muscle.

CONCLUDING REMARKS

Rapid and accurate control of contraction in mammalian skeletal muscle is possible because the voltage sensors/DHPR in the t system in some way directly control the Ca\(^{2+}\)-release channels in the adjacent SR. This tight control of Ca\(^{2+}\) release depends not only on having the skeletal isoform of the DHPR, but also on having a specialized isoform of the Ca\(^{2+}\)-release channel (RyR1) that is potently stimulated by ATP and inhibited by Mg\(^{2+}\). The Mg\(^{2+}\) inhibition makes the Ca\(^{2+}\)-release channels relatively insensitive to activation by cytoplasmic Ca\(^{2+}\) and keeps them closed in a resting fibre. Voltage sensor activation must be able to overcome this inhibitory effect of Mg\(^{2+}\), possibly by lowering the affinity of the release channels for Mg\(^{2+}\). These three primary changes in skeletal muscle are somewhat analogous to the workings of a mouse trap, with all three being necessary for normal function: ATP stimulation is like stretching the spring of the trap, Mg\(^{2+}\) inhibition is like hooking the spring down and the voltage sensor is the trigger that dislodges the hook. Nevertheless, many of the details of the coupling in skeletal muscle are not yet known, for example: (i) precisely how the DHPR physically interact with the RyR; (ii) the exact roles of FKBP12 and triadin; and (iii) the mechanisms involved in termination of Ca\(^{2+}\) release, in particular whether RyR1/Ca\(^{2+}\)-release channels normally inactivate upon activation. Finally, it is interesting to note that some recent studies propose that, under certain circumstances, part of the Ca\(^{2+}\) release in cardiac muscle can be directly controlled by the DHPR like it is in skeletal muscle. Thus, investigation of the exquisite control mechanism underlying E-C coupling in skeletal muscle may actually also shed further light on the coupling in cardiac muscle.

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