3.5. Regulation of RyR1 Activity and Ca^{2+} Release via Interacting Molecules and Channel Modification

The complex structure of the large RyR1 channel complex ensures its sophisticated function in regulating skeletal muscle contractility. RyR1 channels can interact with a row of molecules that coregulate, fine-tune or inhibit the activity of RyR1 and hence the generation of Ca^{2+} gradients in skeletal muscle [55,56]. RyR1 can be subjected to diverse posttranslational modifications (e.g., phosphorylation and nitrosylation) on several residues serving as a feedback system of the local mechano-metabolic environment of the RyR1 channel [57]. The exact and entire mechanisms by which Ca^{2+} release from RyR1 is regulated are highly complex and shall not be discussed in detail in this review. However, the most important interactions and modifications of the RyR1 channel will be highlighted.

In the lumen of the SR, CASQ is the main Ca^{2+} binding protein. However, despite the important role in Ca^{2+} binding, CASQ offers also the ability to influence RyR1 channel activity by mechanical interaction [58]. It has been suggested that dependent on luminal Ca^{2+} content, conformational changes of CASQ in the SR membrane can occur which modulates the molecular interaction with RyR1 in dependence of the luminar Ca^{2+} concentration and by its association with the SR membrane. It has been further shown that CASQ can be phosphorylated and that RyR1 open channel probability is increased in dependence of increased CASQ phosphorylation. Junctin and triadin are important interconnecting proteins between CASQ and RyR1. RyR1 activity is regulated by molecular interaction with Ca^{2+}-dependent CASQ that is anchored to triadin and also the SR membrane [49]. Junctin, with a high sequence homology to triadin, has been found in junctional membranes and seems to serve similar roles as triadin in the SR. Smaller molecules like ATP and Mg^{2+} regulate Ca^{2+} release by binding to the inhibitory I-sites and activating A-sites of the RyR1 complex which modulate channel activity [59]. Mg^{2+} binds preferentially to the inhibitory I-site but also the activating A-site of the RyR1 complex, importantly by competing with Ca^{2+} ions, which bind to this site and serve as strong activators of RyR1. Under resting conditions Mg^{2+} ions act as strong inhibitors of RyR1 channel activity while ATP in contrast increases RyR1 channel activity and Ca^{2+} release. Mg^{2+} ions are often bound to ATP forming an ATP-Mg^{2+} complex. Under conditions of exercise, increasing ATP turnover in skeletal muscle induces the decline in ATP levels and an increase in free Mg^{2+}. This change in metabolic environment acts as a feedback system inducing decreased Ca^{2+} release via RyR1 channel inhibition by Mg^{2+} ions. Besides the acute modulation of RyR1 activity and therefore Ca^{2+} release via changed sarcoplasmatic environment, there are further modifications known that are reversible, more stable and hence provide a timely extended modulation of RyR1 channel activity. For example, chronic adrenergic stimulation has been shown to induce phosphorylation of RyR2 via protein kinase A (PKA) in myocardium but also RyR1 in skeletal muscle [60]. Phosphorylation of RyR1 has been associated with increased fatigability of skeletal muscle due to leaky RyR1 channels and dissociation of FKBP12 [48,57]. In contrast, PKA-dependent phosphorylation of RyR1 has also been shown to be essential for force development of contracting skeletal muscle [61].

3.1. Ca^{2+} Ions and the Initiation of Presynaptic Action Potentials

Ca^{2+} ions are vital for both the complex molecular interactions regulating the shortening of sarcomeres and for the prior electric activation along the motoric endplate that precedes the depolarization of the sarcolemma [4,5]. Upon arrival of an action potential, which is transduced via α-motoneurons of the central nervous system to the presynaptic neuron terminals, voltage dependent Ca^{2+} channels open and induce the Ca^{2+} ion influx from the extracellular space into the cytosol of the presynaptic neuron [6]. Ca^{2+} ions importantly initiate the interaction of acetylcholine containing vesicles with soluble NSF attachment protein receptors (SNARE) allowing these to fuse with the presynaptic membrane and to release acetylcholine into the synaptic cleft [4]. Nicotinic acetylcholine receptors (nAChRs), which also serve as ion channels, are located at the postjunctional folds of the sarcolemma and bind acetylcholine from the synaptic cleft [6,7]. This binding induces activation and opening of the ion channels leading to sodium (Na^+) and potassium (K^+) influx, which causes a local depolarization of the sarcolemma. Depending on the frequency of the incoming impulse pattern this local depolarization of the sarcolemma can be strong enough to cause a voltage-dependent opening of adjacent Na^+ channels at the sarcolemma [8]. This transduces the local depolarization along the sarcolemma towards transverse tubular structures (T-tubuli) which are located in close vicinity to the adjacent terminal cisternae of the SR [9]. At this point, the local depolarization induces a voltage-dependent release of Ca^{2+} ions out of the SR which initiates cross-bridge cycling in myofibrillar compartments

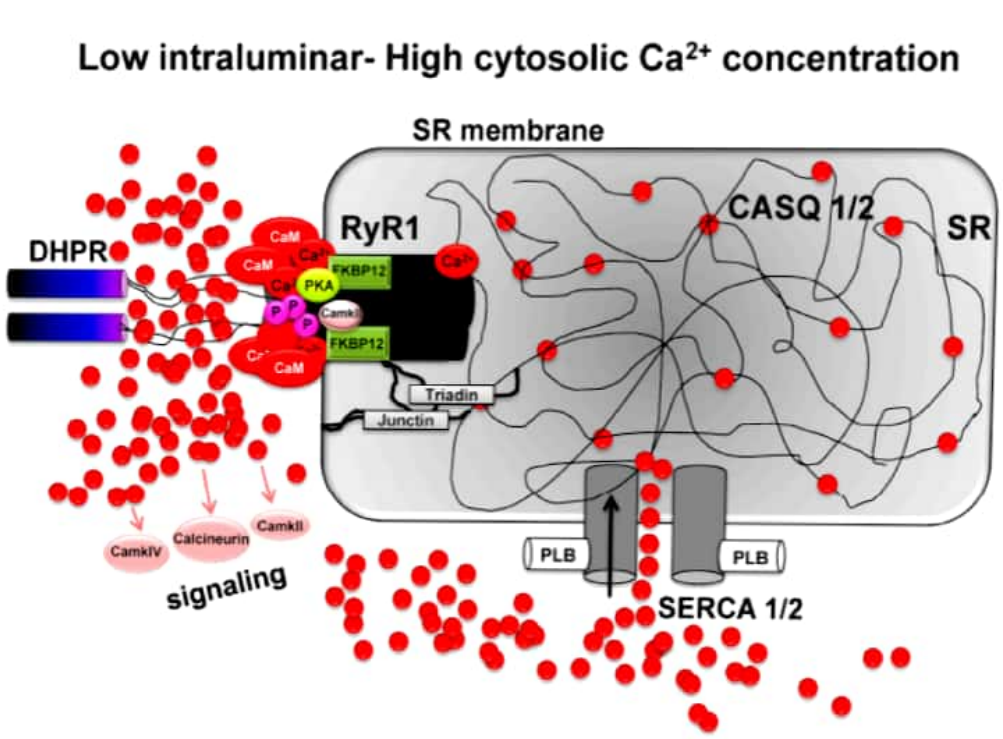

Figure 2. RyR1 channels induce rapid and high gradients in Ca^{2+} ion concentration between luminal compartments of the SR and surrounding sarcoplasm of myofbrills. (**A**) RyR1 opening is primarily controlled by the voltage dependent activation of DHPR which mechanically interacts with RyR1 and regulates channel opening (voltage gating). Further and adjacent RyR1 channels are opened by voltage-independent RyR1-RyR1 interactions (coupled gating) which create locally high Ca^{2+} ion gradients. On the luminar side of the SR, RyR1 channel opening is supported by the combined mechanical interaction with triadin, junctin and the SR membrane. High Ca^{2+} ion concentrations in the SR further supports channel open probability. On the sarcoplasmic side and at low Ca^{2+} concentrations, high amounts of Ca^{2+} unbound apoCaM supports increased open probability and activity of RyR1 while Ca^{2+}-activated CaM inhibits RyR1. PKA and CaMKII have binding sites on RyR1 subunits and are able to phosphorylate RyR1 and modulate channel activity; and (**B**) Upon elevation of sarcoplasmic Ca^{2+} levels due to RyR1 channel opening, decreased Ca^{2+} ion levels in SR inhibit RyR1 channel activity. Sarcoplasmic Ca^{2+} levels increase CaM levels which activate CaMKII, IV and calcineurin. CaM inhibits RyR1 channel activity on the sarcoplasmic side of RyR1 while CaMKII binds to and phosphorylates RyR1. Hyperphosphorylation of RyR1 via PKA and CaMKII may lead to increased dissociation of FKBP12, higher open probability of RyR1 channels and decreased contractility of skeletal muscle under resting conditions. Increased SERCA activity facilitates rapid reuptake of Ca^{2+} ions into the SR.

3.6. Modulation of RyR1-Induced Ca^{2+} Release via Ca^{2+} Ions and Calmodulin

Ca^{2+} ions themselves have a high capability in modulating RyR1 channel activity and, thus, offer an important role in the modulation of their own release via modulation of RyR1 [66]. Ca^{2+}-dependent modulation of the RyR1 channel occurs in different ways. As a direct effect, Ca^{2+} binds either to the high affinity and activating A-site of RyR1, which increases channel activity or the inhibitory I-site which then decreases RyR1 channel conductance. RyR1 is primarily activated at low Ca^{2+} concentration (0.5 µM) and inhibited by elevated concentrations of Ca^{2+} (0.15 mM). Hence, RyR1-induced Ca^{2+} release is directly affected by Ca^{2+} ions via a positive and negative feedback regulation. By this, RyR1 channel conductance is regulated by two distinct Ca^{2+} binding sites on the cytoplasmic domain of the RyR1, controlling and sensing luminal Ca^{2+} flux into modulated RyR1 channel conductance. Typically, at low Ca^{2+} concentration, CaM is preferentially not activated (apoCaM), whereas elevated Ca^{2+} concentration binds to and activates calmodulin (Ca^{2+}-CaM) [67,68]. ApoCaM binds to and activates RyR1, whereas CaM inhibits RyR1 channel conductance. Activated CaM can further activate calmodulin kinase II (CaMKII) which, when activated, phosphorylates RyR1 [9,55]. This can contribute to the discussed increase or decrease in skeletal muscle contractility, which has been observed in response to the phosphorylation of RyR1 channel subunits. Figure 2 illustrates the activity of RyR1 channels upon inhibition and activation by interaction with the most important regulators.

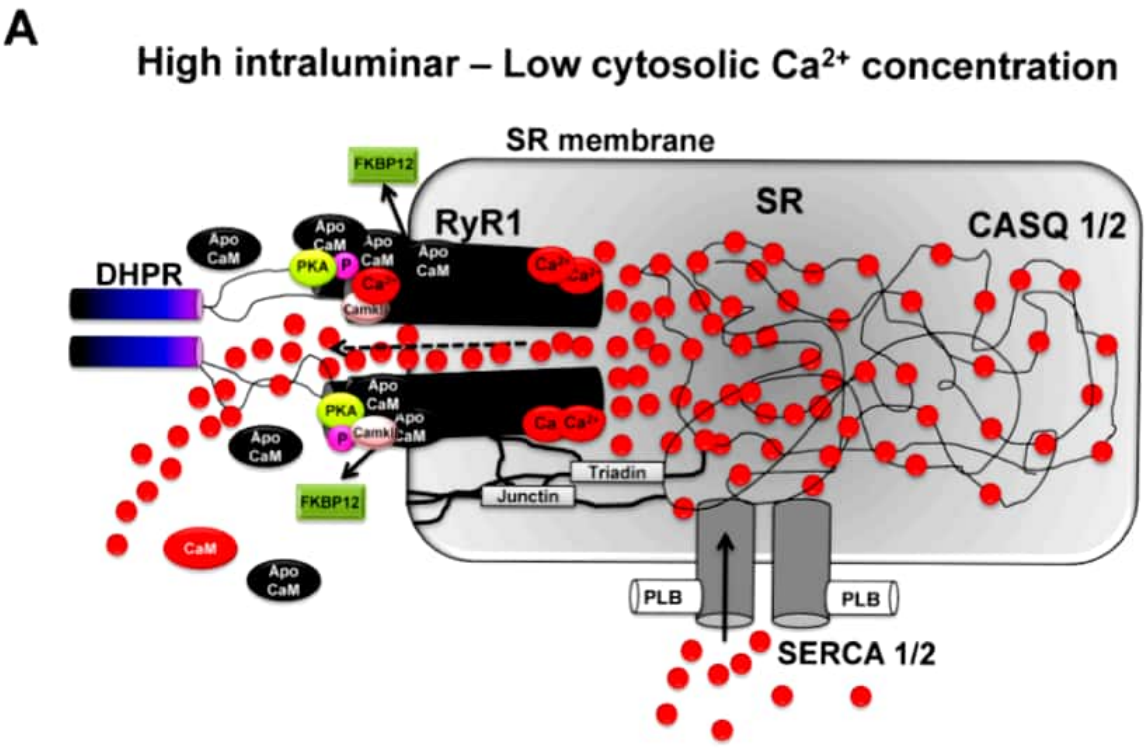

Figure 2. *Cont.*

3.7. Ca^{2+}-Dependent Control of Crossbridge Cycling and Force Generation

The primary force generating mechanism (≈5 pN per sarcomere) depends on the regulated molecular interaction between myosin and actin filaments within sarcomeres. Myosin molecules are composed of two heavy chains (MHC) containing the ATP hydrolyzing head region which binds to actin. Each myosin heavy chain is further associated with two myosin light chains (MLC) binding the

neck region of MHCs. Under conditions of low Ca^{2+} concentrations, the binding sites of the myosin heads on actin filaments are blocked by tropomyosin molecules. Tropomyosin molecules are tightly associated with a troponin complex that contains the subunits troponin T which associates the troponin complex to tropomyosin, the Ca^{2+}-binding and regulatory subunit (troponin C) and troponin I as the inhibitory subunit which blocks myosin binding sites on the actin filaments [69]. Upon Ca^{2+} release via RyR1, two Ca^{2+} ions bind rapidly to troponin C leading to a conformational change within the troponin complex. This induces a release from the inhibitory troponin I subunit from actin, allowing myosin head regions to bind to actin binding sites [70]. At this moment, myosin heads follow the transition from a weak binding state to a strong binding state at which ATP is fully hydrolyzed and inorganic phosphate (Pi) is released. The swinging lever arm action of the myosin head generates the relative movement of thin and thick filaments to each other which, consequently, shortens the sarcomere and generates force. This strong binding state of myosin heads persist until one molecule of ATP is replacing ADP which is still bound to MHC heads [3]. The rate at which the strong binding of myosin head to actin occurs is critically dependent on Ca^{2+} ions bound to troponin C [70]. Thus, myofilament interaction is facilitated by the controlled abundance of Ca^{2+} ions and skeletal muscle force is directly dependent on the Ca^{2+} concentration [71]. No nearby force is generated at very low Ca^{2+} concentration, whereas steadily increasing Ca^{2+} concentrations lead to increased force development of skeletal muscle. This dependency of force development on Ca^{2+} concentration, however, offers a saturation effect where at some point increasing Ca^{2+} levels do not result in further force development of skeletal muscle. The reason for this is that, under rising calcium concentrations, all Ca^{2+}-binding sites on actin are eventually occupied. Although a high Ca^{2+} ion concentration in the cytosol of myofibres is essential for contractility and force development of skeletal muscle, Ca^{2+} can also lead to declined contractility and muscular fatigue. Besides chronically elevated Ca^{2+} levels in myofibrillar compartments e.g., due to leaky RyR1 channels (see Section 3.5), under conditions of severe and repeated muscle contractions, also increased abundance of inorganic phosphate (P_i) and Ca^{2+} ions have been shown to build Ca^{2+}-P_i precipitates [72–74] which blunt skeletal muscle contractility.