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THE ROLE OF ION-REGULATORY MEMBRANE PROTEINS OF EXCITATION-CONTRACTION COUPLING AND RELAXATION IN INHERITED MUSCLE DISEASES

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1. ABSTRACT

The excitation-contraction-relaxation cycle of skeletal muscle fibres depends on the finely tuned interplay between the voltage-sensing dihydropyridine receptor, the junctional ryanodine receptor Ca^{2+}-release channel and the sarcoplasmic reticulum Ca^{2+}-ATPase. Inherited diseases of excitation-contraction coupling and muscle relaxation such as malignant hyperthermia, central core disease, hypokalemic periodic paralysis or Brody disease are caused by mutations in these Ca^{2+}-regulatory elements. Over twenty different mutations in the Ca^{2+}-release channel are associated with susceptibility to the pharmacogenetic disorder malignant hyperthermia. Other mutations in the ryanodine receptor trigger central core disease. Primary abnormalities in the alpha-1 subunit of the dihydropyridine receptor underlie the molecular pathogenesis of both hypokalemic periodic paralysis and certain forms of malignant hyperthermia. Some cases of the muscle relaxation disorder named Brody disease were demonstrated to be based on primary abnormalities in the Ca^{2+}-ATPase. Since a variety of other sarcoplasmic reticulum proteins modulate the activity of the voltage sensor, Ca^{2+}-release channel and ion-binding proteins, mutations in these Ca^{2+}-regulatory muscle components might be the underlying cause for novel, not yet fully characterized, genetic muscle disorders. The cell biological analysis of knock-out mice has been helpful in evaluating the biomedical consequences of defects in ion-regulatory muscle proteins.

2. EXCITATION-CONTRACTION-RELAXATION CYCLE

Contraction is a highly regulated process in mammalian skeletal muscle. Once a sufficient motoneuron activity, above the threshold potential, triggers sarcolemmal depolarization via the neuromuscular junction, an action potential travels along the muscle surface membrane and enters the transverse tubular system, also referred to as t-tubules. At specialized junctions, called triads, the signal is transmitted from the t-tubules to the terminal cisternae, thus causing the release of Ca^{2+}-ions from the sarcoplasmic reticulum (SR) which in turn activates the contractile apparatus. Binding of Ca^{2+} to the troponin complex alters the interactions between tropomyosin and the contractile machinery allowing the proper interaction between actin molecules and myosin heads. Thus muscle contraction occurs via myofilament sliding (1).

The release of intracellular Ca^{2+} in response to membrane depolarization is a key step of excitation-contraction coupling. Two large membrane protein complexes are mainly involved in this regulatory process, the voltage-sensing dihydropyridine receptor (DHPR) located in the junctional t-tubules and the ryanodine receptor (RyR) of the junctional SR. According to our present understanding, the alpha-1 subunit of the DHPR is activated by membrane depolarization and then interacts directly with the RyR which in turn releases Ca^{2+}-ions into

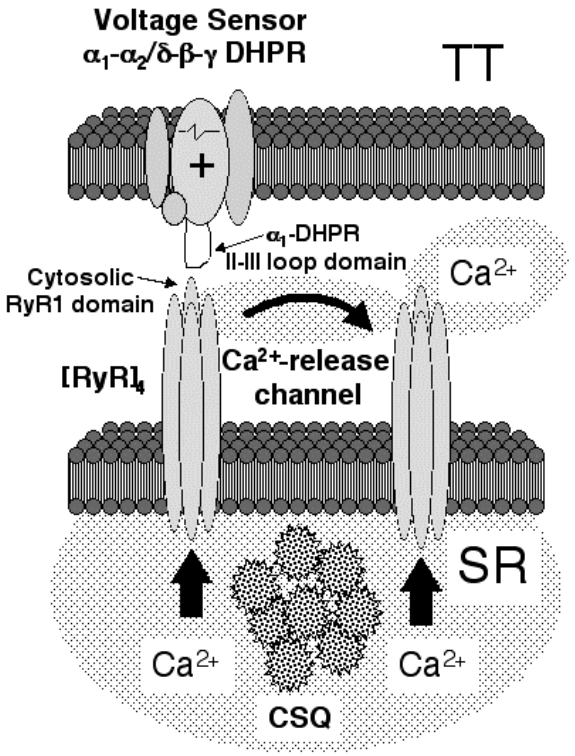

Figure 1. Overview of triadic signal transduction process underlying excitation-contraction coupling in skeletal muscle fibres. Changes in membrane polarity are sensed by the alpha-1 dihydropyridine receptor (DHPR) and are followed by conformational changes in the transverse tubular (TT) receptor which allow its II-III loop domain to directly interact with a cytoplasmic domain of the ryanodine receptor (RyR) of the junctional sarcoplasmic reticulum (SR). Transient opening of the Ca^{2+}-release channel triggers a massive release of Ca^{2+}-ions into the cytosol. Subsequently, excessive local Ca^{2+}-ions probably activate non-junctional RyRs, which are not directly coupled to the voltage sensor. Clusters of the high-capacity Ca^{2+}-binding protein calsequestrin (CSQ) provide a junctional SR Ca^{2+}-pool for fast ion release and can therefore be considered positive regulators of the RyR. Once the Ca^{2+}-signal reaches the myofibrillar apparatus, the second messenger binds to troponin c (TnC) and thereby allows a proper interaction between actin and myosin filaments resulting in muscle contraction.

the cytosol (figure 1). Not all RyRs are coupled to a set of DHPRs, some single receptors are found in the SR membrane between triad junctions. These Ca^{2+}-channels are probably activated by excess Ca^{2+}, released from neighboring RyRs thereby amplifying and propagating the Ca^{2+}-signal (2). This mechanism is referred to as Ca^{2+}-induced Ca^{2+}-release and seems to be the dominant activation pathway in cardiac muscle (3). This review will focus on the skeletal muscle type of excitation-contraction coupling and muscle relaxation. Other membrane components found in the SR, e.g. the Ca^{2+}-storing protein calsequestrin (CSQ) and the junctional element triadin (TRI) are also thought to be involved in the regulation of Ca^{2+}-homeostasis by influencing the opening time of the RyR. To reverse the Ca^{2+}-signal and in order for muscle relaxation to occur, Ca^{2+}-ions are pumped back into the SR lumen in an energy-dependent manner. This is primarily accomplished by ATP driven Ca^{2+}-pumps of the SERCA type (4). In contrast, in cardiac muscle, the Na^{+}/Ca^{2+}-exchanger appears to be the major Ca^{2+}-removal system. In skeletal muscle the role of this ion-exchanger, which is indirectly driven by the pump action of the surface Na^{+}/K^{+}-ATPase, is not fully understood.

2.1.Dihydropyridine receptor

The dihydropyridine receptor (DHPR) is a voltage-dependent L-type Ca^{2+}-channel that consists of five subunits: alpha-1 of 175 kDa, alpha-2/delta of 143/27 kDa, beta of 54 kDa, and gamma of apparent 30 kDa. With its four domains each consisting of six loops that span the cell membrane, alpha-1 is the largest of the subunits. Being the principal functional DHPR-subunit, alpha-1 exhibits the pharmacological binding sites for numerous agonists and antagonists, forms the Ca^{2+}-channel pore and represents the voltage-sensing unit of the transverse-tubular receptor complex (5). The other subunits have regulatory functions (6, 7). They help in targeting the alpha-1 subunit to the t-tubules and enhance their ability to sense depolarisations or to act as a Ca^{2+}-channel (6, 7). In skeletal muscle, the beta subunit seems to have an important function because mice lacking this subunit die at birth from asphyxia and electrical stimulation of beta-null myotubes fails to induce contraction (8).

Depending on the skeletal or cardiac isoform, the alpha-1 subunit acts as a voltage sensor and/or Ca^{2+}-channel. The importance of DHPR for excitation-contraction coupling is clearly demonstrated by experimental evidence from mice suffering from muscular dysgenesis (9). These animals have a point mutation in the alpha-1 subunit of the receptor complex resulting in a lack of charge movement and interruption of excitation-contraction coupling. A proper signal transduction process could be restored by injecting alpha-1 DHPR into dysgenic mouse muscle (9). Voltage-sensing, however, is only restored by replacement of the skeletal muscle isoform. Using chimeric DHPR constructs, it could be shown that the cytoplasmic II-III loop domain of the alpha-1 DHPR is extremely critical for the interaction with the RyR (10). Upon membrane depolarization, the DHPR undergoes a conformational change, which allows a short stretch of primary sequence in the II-III loop domain (11) to interact with a corresponding cytoplasmic domain of the RyR (12), and thereby triggers Ca^{2+}-release (figure 1). Interactions between RyR and the III-IV loop domain of the alpha-1 DHPR might be responsible for ending the Ca^{2+}-signaling process (13).

2.2. Ryanodine receptor

Ryanodine receptors (RyR) represent a family of intracellular Ca^{2+}-release channels that play important roles in signal transduction events and the maintenance of overall ion homeostasis. Three isoforms encoded by different genes have been described (14). RyR1 and RyR2 are the main isoforms, found in skeletal and cardiac muscle

respectively, while RyR3 has a wider tissue distribution including non-muscle tissues such as the brain. The generation of knock-out mice, either deficient in RyR1 or RyR2, confirmed the crucial role of the Ca^{2+}-release channel for excitation-contraction coupling. Mice deficient in RyR1 died perinatally of respiratory failure (15) while RyR2 deficient mice died at embryonic day 10 with morphological abnormalities of the heart tube (16). The role of RyR3 in regulating Ca^{2+}-homeostasis is controversial, since mice with a targeted mutation in RyR3 do not exhibit any gross skeletal muscle abnormality (17). On the other hand, studies on contractile properties of postnatal RyR3 knock-out muscles revealed that the amount of force generated by electrical or caffeine-induced stimulation was strongly reduced in these skeletal muscles fibres (18). Since RyR3 is mainly present in developing skeletal muscle, this isoform of the SR Ca^{2+}-release channel may be important for Ca^{2+}-regulation in newborns.

RyRs are extremely large proteins consisting of identical subunits of 565 kDa (14). Electron microscopical analyses (19) and chemical crosslinking studies (20) imply a predominantly tetrameric structure for the skeletal muscle RyR isoform with the hydrophobic parts of the four subunits forming a membrane-spanning base plate and the more hydrophilic segments forming a cytoplasmic domain which bridges the gap between the t-tubular and SR membranes (21). Major conformational changes accompany the opening and closing of the Ca^{2+}-channel whereby numerous loosely packed structural domains of the cytoplasmic assembly of the RyR seem to be responsible for maintaining the structural integrity of the junctional coupling and allow Ca^{2+}-ions to freely diffuse through the receptor (21).

Tightly associated with RyR is the immunophilin protein named FKBP12 that binds the immuno-suppressive drug FK-506 (22). Co-immunoprecipitation experiments confirmed that one FKBP12 molecule is bound to each RyR monomer. The RyR2 isoforms binds additionally FKBP12.6. The opening of the Ca^{2+}-release channel is modulated by FKPB12 which could be shown by protein depletion experiments (22). Mutant mice deficient in FKBP12 have apparently normal skeletal muscle but exhibit severe dilated cardiomyopathy (23) suggesting that FKBP12 is more important in the heart than in skeletal muscle fibres. Depletion of FKBP12 increases Ca^{2+}-currents resulting in apparently leaky ion channels. So far no mutations in the gene encoding FKBP12 could be linked to any of the known muscle diseases. Another regulatory protein of the SR Ca^{2+}-release channel is the ubiquitous 17 kDa Ca^{2+}-binding protein named calmodulin (CaM). Directly associated with the RyR, CaM modulates the open time of the cardiac and skeletal muscle isoform of the RyR (24). Low concentrations of CaM activate the channel while high concentrations inhibit channel function.

2.3. Sarcoplasmic reticulum regulatory proteins

The SR contains a number of proteins which are directly or indirectly involved in the regulation of Ca^{2+}-homeostasis and maintenance of triad receptor interactions and thus influence the activity of the RyR. Stimulation-induced Ca^{2+}-release through the RyR complex is dependent on the overall ion-loading of the SR, which is largely dependent on high-capacity Ca^{2+}-binding proteins. Calsequestrin (CSQ), a relatively low-affinity Ca^{2+}-storage protein with many ion-binding sites (25), forms a matrix in the lumen of the junctional terminal cisternae where it represents the major Ca^{2+}-reservoir complex (26). In addition to the 63 kDa CSQ monomer, post-translationally modified isoforms of approximately 120 to 200 kDa exist in adult skeletal muscle fibres (20). The exact functional role of these CSQ-like proteins in Ca^{2+}-homeostasis is not well understood. It has been suggested that calsequestrin clusters modulate Ca^{2+}-channel properties (27) because the opening of the RyR is preceded by a release of Ca^{2+}-ions from CSQ which raises the intralumenal free Ca^{2+}-concentration (28). Affinity chromatography and overlay assays showed that CSQ and the CSQ-binding protein junctin (JN), together with the RyR, form high-molecular-mass complexes in the junctional SR (29). This suggests the presence of tight protein-protein-interactions in the SR and possibly CSQ binds directly, or via JN, to the RyR and hence indirectly regulates the opening and closing of the Ca^{2+}-release channel. Structurally related to CSQ is the Ca^{2+}-binding component calreticulin (CAL) of apparent 60 kDa, whose exact role in skeletal muscle Ca^{2+}-sequestration and -release is not quite clear. CAL is highly expressed in developing skeletal muscle (30, 31) and is also present in many mature non-skeletal/cardiac muscle tissues. Besides its role as a Ca^{2+}-reservoir protein, CAL is also involved in protein folding, cell adhesion and cardiac development (32). In the longitudinal SR lumen, the existence of two further Ca^{2+}-binding proteins, sarcalumenin (SAR) and the histidine-rich Ca^{2+}-binding protein (HCP), was demonstrated. The RyR is modulated by phosphorylation/ dephosphorylation of SAR and HCP (33).

The 94 kDa glycoprotein triadin (TRI) is an abundant component of the junctional face membrane and may play an important role in triad architecture and/or regulation of Ca^{2+}-homeostasis (34-36). Recent data suggest a negative regulatory role of TRI on the Ca^{2+}-release channel, while CSQ functions as an endogenous activator of RyR-mediated Ca^{2+}-release (37). Extensive crosslinking experiments showed that the alpha-1 DHPR, the RyR and CSQ form high-molecular-mass complexes and are therefore most likely closely associated in a gigantic triad complex (38, 39). However, TRI is not present in crosslinking-induced complexes indicating that it is not directly coupled to the RyR. Diagonal non-reducing/reducing two-dimensional gel electrophoresis showed that TRI aggregates with itself under native conditions and that these complexes are in close neighborhood relationship to the RyR, but are not directly coupled to the Ca^{2+}-release channel complex (40). Possibly the central function of TRI clusters is the maintenance of the structural integrity of the triad junction. TRI might thus only indirectly participate in the regulation of the excitation-contraction-relaxation cycle. In addition to TRI, a distinct 90 kDa junctional face protein (JFP) has also been identified in triad preparations (41). The 90 kDa JFP appears to be tightly associated with the RyR and alpha-1 DHPR complex, suggesting a possible role in the regulation of excitation-contraction coupling and Ca^{2+}-handling (42).

Table 1. Established primary abnormalities in the ryanodine receptor (RyR) Ca^{2+}-release channel, the voltage-sensing dihydropyridine receptor (DHPR) and the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), associated with inherited muscle diseases of the excitation-contraction-relaxation cycle.

Inherited muscle disease	Gene	Mutation
Malignant hyperthermia susceptibility	RyR1	N-terminal RyR1-Region 1
Malignant hyperthermia susceptibility	RyR1	Central RyR1-Region 2
Malignant hyperthermia susceptibility	RyR1	C-terminal RyR1-Region 3
Malignant hyperthermia susceptibility	CACNA1S	Alpha-1 DHPR (III-IV loop)
Malignant hyperthermia susceptibility	CACNA2	Alpha-1/delta DHPR
Central core disease	RyR1	Various RyR domains
Hypokalemic period paralysis	CACNA1S	Alpha-1 DHPR
Brody disease	ATP2A1	SERCA1 Ca^{2+}-ATPase

The major function of the sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPases (SERCA) of the longitudinal tubules is to pump Ca^{2+}-ions back into the SR lumen to prevent a prolonged refractoriness phase in the excitability of muscle cells and to furthermore eliminate potential Ca^{2+}-induced metabolic disturbances and/or cellular damages. The energy-dependent removal of Ca^{2+}-ions allows for the re-establishment of a tropomyosin-mediated inhibition of filament sliding and therefore initiates muscle relaxation. Mammalian Ca^{2+}-ATPases are encoded by three separate genes. SERCA1 is present in fast-twitch skeletal muscle while SERCA2 is found in cardiac and slow-twitch muscles (43). In native membrane vesicles, both the fast and slow isoforms of the SR Ca^{2+}-ATPase form predominantly tetrameric structures (39, 44). Although detergent-solubilized SERCA monomers were shown to be capable of performing all the required enzymatic steps to induce muscle relaxation (45), the physiologically active pump units are believed to be represented by oligomeric complexes of the SR Ca^{2+}-ATPase. Direct protein-protein interactions between 110 kDa SERCA subunits are postulated to be involved in co-operative enzyme kinetics and protection against proteolytic degradation (46). A diagramatic overview of the various Ca^{2+}-regulatory components involved in excitation-contraction coupling and muscle relaxation is given in Figure 2.

3. HEREDITARY MUSCLE DISEASES

Muscle diseases can be caused by alterations in structural, metabolic or contractile proteins (47). Severe pathophysiological effects are caused by mutations of proteins directly involved in the excitation-contraction-relaxation cycle, e.g. membrane elements involved in voltage-sensing, signal transduction, Ca^{2+}-release and Ca^{2+}-uptake (48-51). Mutations in the skeletal muscle RyR clearly cause disturbances in Ca^{2+}-homeostasis. The mutated RyR1 isoform is the primary abnormality responsible for excessive Ca^{2+}-release in central core disease. Genetic susceptibility to episodes of the pharmacogenetic disorder malignant hyperthermia is also based on changes in the primary structure of the Ca^{2+}-release channel. However, malignant hyperthermia is clearly heterogeneous, since mutations in the voltage-sensing alpha-1 subunit of the DHPR have also been linked to it. Hypokalemic periodic paralysis is associated with mutations in the gene associated with the transverse tubular voltage-sensor. Inherited diseases linked to mutations in genes encoding for ion channels are therefore now referred to as channelopathies. Primary abnormalities in SERCA1 were shown to be responsible for certain cases of Brody disease, a disorder of impaired muscle relaxation. Table 1 lists established Ca^{2+}-handling proteins involved in the excitation-contraction-relaxation cycle which play a primary role in the molecular pathogenesis of genetic muscle diseases.

3.1. Malignant Hyperthermia

Malignant hyperthermia (MH) is an autosomal dominantly inherited predisposition of otherwise healthy people who undergo an uncontrollable skeletal muscle hypermetabolism when exposed to volatile anesthetics or muscle relaxants (52). The incidence of MH during anesthesia is about 1 in 15,000 in children and about 1 in 50,000 to 1 in 100,000 in adults (53). These numbers may underestimate the incidence because only a small number of MH-susceptible persons undergo general anesthesia with MH-triggering agents. In addition, some MH patients experience only mild reactions to MH-causing anesthetics, which are often difficult to detect. Well known triggering substances are the volatile drug halothane and the depolarizing muscle relaxant succinylcholine (containing the preservative 4-chloro-m-cresol). Both drugs may cause an excessive release of Ca^{2+}-ions from the SR lumen into the cytosol thereby triggering sustained muscle contracture (54). The clinical symptoms of MH are muscle rigidity, hyperthermia associated with acidosis, hyperkalemia, and hypoxia. If not treated immediately with the highly effective Ca^{2+}-release inhibitor dantrolene, up to 70 % of MH episodes result in fatalities. Although MH is primarily a metabolic disorder of skeletal muscle, secondary pathophysiological changes also occur in the heart, kidneys and the lungs. Following the application of dantrolene, secondary treatment to counteract MH symptoms involve discontinuing general anesthesia, supporting cardiovascular function, correcting hypoxia and reducing body temperature through artificial cooling.

Genetic linkage analysis has revealed several MH hotspots on the RyR1 gene in human chromosomes. Initially mutations associated with human MH were located to the gene encoding for the skeletal muscle RyR1 isoform of the SR Ca^{2+}-release channel (55-57). The mutated RyR1 is believed to exhibit a prolonged ion channel opening time which causes a transient increase in cytosolic Ca^{2+}-levels during excitation. An excessive cytosolic Ca^{2+}-

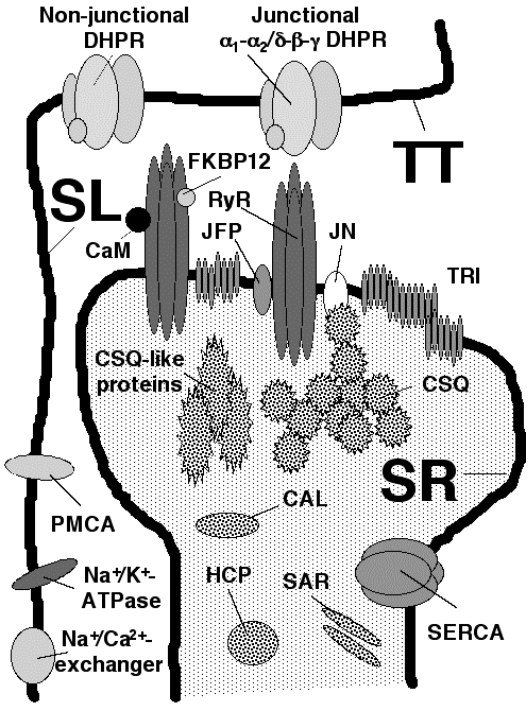

Figure 2. Diagramatic presentation of skeletal muscle proteins involved in the excitation-contraction-relaxation cycle. Triads are composed of transverse tubules (TT) and two surrounding terminal cisternae of the sarcoplasmic reticulum (SR). Located in the TT is the multimeric dihydropyridine receptor (DHPR) with its five subunits, the voltage-sensing alpha-1 subunit, the transmembrane alpha-2/delta and gamma subunits, as well as the cytosolic beta subunit. The ryanodine receptor (RyR) Ca^{2+}-release channel is enriched in the junctional SR membrane. Tightly associated with the receptor are two auxiliary proteins, the immunophilin protein of 12 kDa (FKBP12) and the Ca^{2+}-binding component calmodulin (CaM), which both modulate the opening time of the RyR. The Ca^{2+}-binding proteins calsequestrin (CSQ), calreticulin (CAL), sarcalumenin (SAR) and histidine-rich Ca^{2+}-binding protein (HCP) are thought to be involved in Ca^{2+}-storage and the fine regulation of the RyR. Junctin (JN) represents a CSQ-binding protein. Triadin (TRI) clusters and the 90 kDa junctional face protein (JFP) are probably involved in maintaining receptor interactions and the overall architectural arrangement of triad junctions. The SR Ca^{2+}-ATPase (SERCA) represents the major energy-dependent Ca^{2+}-reuptake mechanism in skeletal muscle fibres and this enzyme is responsible for initiating the muscle relaxation step. In addition, a surface Ca^{2+}-pump (PMCA) and a Na^+/Ca^{2+}-exchanger, which is indirectly driven by the sarcolemmal Na^+/K^+-ATPase, exist in skeletal muscle. Complex interactions between these various Ca^{2+}-channels, Ca^{2+}-binding proteins and Ca^{2+}-pumps provide the molecular basis which regulates muscle Ca^{2+}-homeostasis.

concentration leads to glycogenolysis, ATP depletion, mitochondrial oxidation, production of excess lactic acid and CO_2 and ultimately to a disturbance of intra- and extracellular ion homeostasis with consequent muscle cell damage (52). So far 22 different MH mutations have been found on chromosome 19q13.1 encoding for the Ca^{2+}-release channel. Clustering of primary abnormalities occurs within the myoplasmic RyR1-domain at the central receptor region (Region 2; 12 mutations) and at the extreme N-terminal region (Region 1; 9 mutations). The only known exception to these hotspot mutations is a single MH-causing mutation in the extreme C-terminal region (RyR1-Region 3). However, these mutations are considered to be responsible for only approximately 50 % of primary abnormalities in the entire MH population. Further linkage analysis identified five additional possible locations for mutations. The most important ones are linked to the alpha-1 and the alpha-2/delta subunits of the DHPR. So far two mutations have been found within the gene encoding the alpha-1 DHPR (51, 58). This is interesting because the alpha-1 subunit is thought to directly interact with the SR RyR during excitation-contraction coupling. A mutated alpha-1 DHPR might therefore modify the interaction between the two receptors and thereby delay or inhibit the closure of the RyR with the consequence of excessive Ca^{2+}-release (59). Most of the unknown MH mutations are believed to be present in the RyR1 gene. On the other hand, it can not be excluded that primary abnormalities in other Ca^{2+}-handling proteins besides the RyR and DHPR are also associated with MH. However, at the current stage of MH research, one can preliminarily classify this pharmacogentic disease as a channelopathy of excitation-contraction coupling.

Genetic susceptibility to MH is also present in pigs, the so-called porcine stress syndrome (PSS) (60). Crises of PSS are brought on by physical and emotional stresses, including overheating, exercise, mating, transportation and fear. Muscle rigidity, tachycardia and fever characterize the attacks. PSS is therefore a highly suitable animal model for human MH. In pigs it is caused by a single point mutation in the RyR1 gene (60) which occurs predominantly with homozygous animals. Recent studies have shown that external triggering substances or certain environmental circumstances can also evoke PSS/MH attacks in heterozygous animals (61). Experimental evidence from halothane-treated rabbit muscle suggest that this drug might trigger abnormal Ca^{2+}-homeostasis in MH via oligomerization of the mutated RyR. Halothane clearly induces oligomerization of the Ca^{2+}-binding protein CSQ, the junctional Ca^{2+}-release channel and the alpha-1 DHPR (62). Possibly a modulation of Ca^{2+}-release activity by halothane is mediated by direct interactions between the voltage sensor, the RyR and the luminal Ca^{2+}-reservoir which could then trigger a rapid release of Ca^{2+}-ions. Recently, native gel analysis showed that halothane induces an isoform-specific oligomerization of the Ca^{2+}-release channel, since the cardiac RyR-2 isoform was not affected by the drug (63). Hence, certain MH-triggering substances are not only able to directly influence protein-protein interactions within muscle membrane complexes, but also appear to distinguish between differing isoform configurations.

In the absence of a clear family history of MH episodes, susceptibility to MH is usually tested on fresh

biopsies of muscle bundles with an *in vitro* contracture test. The test is based on the property of MH muscle to be abnormally sensitive to contraction-inducing agents, e.g. halothane and caffeine (64, 65). The test is also positive for patients with MH-associated diseases such as central core disease (66), King-Denborough syndrome (67) and Evans myopathy. Though the underlying mechanism is most likely different, MH-like episodes might occur in Duchenne muscular dystrophy (68), myotonia fluctans (69), myotonia congenita (70) and other myopathies (51).

3.2. Central Core Disease

Closely related to MH is central core disease (CCD), which is caused by a mutation in the skeletal muscle Ca^{2+}-release channel. Four mutations in the gene encoding RyR1 have been linked to this rare autosomal-dominant inherited muscle disease (71, 72). CCD is non-progressive and characterized by hypotonia, delayed motor development, and muscle weakness. The onset of the disease is early in childhood with hypotonia (floppy infant syndrome), but muscle strength usually improves during life especially after continuous exercise. Characteristic for CCD are areas of unstructured myofibrils lacking mitochondria (73). Therefore a lack of oxidative enzymes in the central regions of skeletal muscle cells (74) and structural disintegration of the contractile apparatus is employed in the diagnosis of CCD. It has been proposed that myoplasmic Ca^{2+}-overload due to a mutation in RyR1 is responsible for the mitochondrial damage. Decreased metabolic activity might therefore be the reason for the disorganization of the contractile apparatus. If the RyR is defective, the intracellular Ca^{2+}-level should be elevated and the SERCA type Ca^{2+}-pumps and the surface Na^{+}/Ca^{2+}-exchanger should have a higher workload in removing excess cytosolic Ca^{2+}-ions. If the Ca^{2+}-concentration rises to very high levels, the mitochondria should participate in Ca^{2+}-removal from central areas and could possibly be damaged in the effort to protect the cell from Ca^{2+}-induced cell necrosis (75). Loss of mitochondria from the center of the cell should lead to a reduction in ATP production and might be the underlying cause of the muscle weakness observed in central core disease.

3.3. Hypokalemic periodic paralysis

Hypokalemic periodic paralysis (HypoPP) is a rare autosomal dominant inherited disorder, with a frequency of 1:100,000, that is associated with muscle weakness and low K^{+}-levels. Episodes occur often late at night or early in the morning after consumption of carbohydrate-rich meals or strenuous physical activity (76, 77). Initially affected are proximal lower extremities but symptoms may progress to all four limbs and the trunk. In contrast, bulbar, respiratory and cardiac muscle are unaffected. Acute episodes are treated with oral or intravenous K^{+}-repletion. Otherwise avoidance of triggering factors, administration of oral K^{+}-supplements and carbonic anhydrase inhibitors are used as prophylaxis.

Gene analysis of families affected with HypoPP showed that the disorder is associated with mutations in the gene encoding the voltage-sensing skeletal muscle alpha-1 subunit of the DHPR (78-80). The described point mutations are located within the highly conserved S4 regions of the repeats II and IV of the Ca^{2+}-channel which might alter interactions with the RyR and thus interfere with excitation-contraction coupling (81). Whether the mutation of the alpha-1 DHPR is responsible for alterations in the excitation-contraction-relaxation cycle is still unclear. Cultured myotubes from patients with HyopPP showed a reduction in DHP-sensitive Ca^{2+}-currents (82) and slower activation of the DHPR Ca^{2+}-channel (83). It is possible that changes in cytosolic Ca^{2+}-levels could inactivate sarcolemmal and t-tubular Na^{+}-channels and so reduce K^{+}-fluxes resulting in membrane depolarization and hypokalemia.

3.4. Brody disease

In 1969 Brody (84) described a muscle disorder with painless muscle cramps and exercise-induced impairment of muscle relaxation. Biochemical and immunocytochemical studies showed a significant decrease in Ca^{2+}-uptake and Ca^{2+}-ATPase activities in SR vesicles isolated from patients with Brody disease (85, 86). Although the Ca^{2+}-ATPase activity is reduced by about 50 %, the total number of Ca^{2+}-pump units appear to be normal (87). Muscle from patients with this disorder show a slow restoration of the cytosolic Ca^{2+}-concentration and muscle relaxation, which may explain the clinical symptoms of stiffness and cramps. The administration of dantrolene, which reduces the cytosolic Ca^{2+}-concentration by blocking the RyR-mediated Ca^{2+}-release from the SR lumen, is beneficial in the treatment of Brody disease (88). Karpati et al. (86) found that the fast-twitch muscle fibers were mainly affected, leading to the prediction that the gene encoding the SERCA1 could be mutated. Odermatt et al. (89) described several mutations in the ATP2A1 gene confirming the SERCA1 as the affected protein at least in a subpopulation of patients with Brody disease. On the other hand, Zhang et al. (90) could eliminate the SERCA1 isoform as a candidate for Brody disease in three patients showing the above described clinical symptoms. Therefore Brody disease appears to be a genetically heterogeneous disease in which other factors, i.e. regulators of SERCA pump units, may also be involved.

4. CONCLUSIONS AND PERSPECTIVES

The excitation-contraction-relaxation cycle is regulated by a complex signal transduction mechanism using fluctuations in the cytoslic Ca^{2+}-concentration as a second messenger system. The major Ca^{2+}-regulatory membrane proteins involved in this process have been identified. The skeletal muscle alpha-1 subunit of the transverse tubular DHPR is activated by changes in membrane polarity and interacts via its II-III loop domain with the junctional SR RyR, which in turn releases Ca^{2+}-ions that trigger contraction via binding to the troponin/tropomyosin system. SR Ca^{2+}-ATPases of the longitudinal tubules are responsible for re-establishing Ca^{2+}-homeostasis by pumping Ca^{2+}-ions back into the SR lumen where it is stored by CSQ and other Ca^{2+}-binding proteins. How exactly these proteins interact during excitation-contraction coupling and muscle relaxation is not fully understood as yet. Electron microscopical studies,

domain binding experiments and chemical crosslinking experiments have shown that direct protein-protein interactions are very important for signal transduction at the triad and that the RyR Ca^{2+}-release channel is modulated by a variety of factors. With the continuous identification and biochemical characterization of novel triad proteins, the number of modulating components will surely increase. Mice deficient in the major membrane elements involved in the excitation-contraction-relaxation cycle have been and will be extremely useful in evaluating the importance of individual SR proteins. Disturbances in the interplay between ion-regulatory proteins clearly result in abnormal Ca^{2+}-homeostasis which can cause serious muscle diseases. Mutations in the genes encoding the RyR1, the alpha-1 DHPR and SERCA 1 could be linked to malignant hyperthermia, central core disease, hypokalemic periodic paralysis or Brody disease. So far no mutations in genes encoding CSQ or TRI have been linked to any existing inherited muscular or neurological disorder. However, experimental over-expression or loss of these proteins in animal models demonstrates that potential primary abnormalities in key Ca^{2+}-regulatory muscle proteins causes severe disturbances in excitation-contraction coupling. In future, modern genetic screening approaches and improved molecular biological techniques will certainly identify novel disease genes at an even faster rate than today. However, only a well designed inter-disciplinary approach on the part of all biomedical research fields will help to fully elucidate the molecular and cellular mechanisms of inherited diseases. Based on such knowledge, rationale drug development, myoblast transfer or gene therapy can be properly designed in order to counteract the severe symptoms of inherited human muscle disorders.

5. ACKNOWLEDGMENTS

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Abbreviations: CAL: calreticulin, CaM: calmodulin, CCD: central core disease, CSQ: calsequestrin, DHPR: dihydropyridine receptor, FKBP12: immunophilin protein of 12 kDa, HCP: histidine-rich calcium-binding protein, HypoPP: hypokalemic periodic paralysis, JFP: junctional face protein, JN: junctin, MH: malignant hyperthermia, PMCA: plasma membrane calcium adenosine triphosphatase, PSS: porcine stress syndrome, RyR: ryanodine receptor, SAR: sarcalumenin, SERCA: sarcoplasmic or endoplasmic calcium adenosine triphosphatase, SL: sarcolemma, SR: sarcoplasmic reticulum, TnC: troponin c, TRI: triadin, TT: transverse tubules

Key Words: Excitation-Contraction Coupling, Ryanodine Receptor, Dihydropyridine Receptor, Malignant Hyperthermia, Central Core Disease, Hypokalemic Periodic Paralysis, Review

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