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Divergent biophysical properties, gating mechanisms, and possible functions of the two skeletal muscle $Ca_V1.1$ calcium channel splice variants

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Abstract

Voltage-gated calcium channels are multi-subunit protein complexes that specifically allow calcium ions to enter the cell in response to membrane depolarization. But, for many years it seemed that the skeletal muscle calcium channel $Ca_V1.1$ is the exception. The classical splice variant $Ca_V1.1a$ activates slowly, has a very small current amplitude and poor voltage sensitivity. In fact adult muscle fibers work perfectly well even in the absence of calcium influx. Recently a new splice variant of the skeletal muscle calcium channel $Ca_V1.1e$ has been characterized. The lack of the 19 amino acid exon 29 in this splice variant results in a rapidly activating calcium channel with high current amplitude and good voltage sensitivity. $Ca_V1.1e$ is the dominant channel in embryonic muscle, where the expression of this high calcium-conducting $Ca_V1.1$ isoform readily explains developmental processes depending on L-type calcium currents. Moreover, the availability of these two structurally similar but functionally distinct channel variants facilitates the analysis of the molecular mechanisms underlying the unique current properties of the classical $Ca_V1.1a$ channel.

Keywords

$Ca_V1.1$; Voltage-gated calcium channel; L-type calcium current; Skeletal muscle; Excitation–contraction coupling

Function of voltage-gated calcium channels in skeletal muscle EC coupling

In excitable cells, voltage-gated calcium channels convert membrane depolarization into intracellular calcium signals which regulate contraction, secretion, and transcription. In addition, calcium entering through voltage-gated calcium channels is important for many

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processes during the development of nerve and muscle cells. Accordingly, altered calcium channel functions cause diseases and voltage-gated calcium channels are prime drugs targets for the treatment of cardio-vascular and neurological dysfunctions.

In skeletal muscle the voltage-gated calcium channel $Ca_V1.1$ is the primary voltage-sensor in EC coupling (Melzer et al. 1995). In response to membrane depolarization $Ca_V1.1$ activates calcium release from the sarcoplasmic reticulum through the ryanodine receptor (RyR1). This process is independent of calcium influx (Armstrong et al. 1972; Rios and Brum 1987), but requires the exact localization of $Ca_V1.1$ and its physical interaction with the calcium release channel (Block et al. 1988; Grabner et al. 1999; Kugler et al. 2004). Conversely, a retrograde interaction between the RyR1 and $Ca_V1.1$ results in an amplification of the L-type calcium current (Grabner et al. 1999; Nakai et al. 1996). Nevertheless, the classical skeletal muscle L-type calcium currents are small, activate slowly and open only at 30 mV more positive membrane potentials than EC coupling. Here we contrast the biophysical properties of the adult $Ca_V1.1a$ (Flucher and Tuluc 2011) and the recently discovered embryonic $Ca_V1.1e$ (Tuluc et al. 2009; Flucher and Tuluc 2011) isoforms and thus review the evidence for the molecular mechanisms responsible for the specific gating properties of $Ca_V1.1$ and their physiological implications.

The specific gating behavior of $Ca_V1.1$ is determined by different domains in the channel's primary structure

The α_1 subunit of voltage-gated calcium channels contains four homologous repeats (I–IV) each formed by six transmembrane segments (S1–S6) (Fig. 1a). Similar to other voltage-gated ion channels, the transmembrane segments S1–S4 (Fig. 1a blue segments) form the voltage sensor with S4 containing positively charged amino-acids every three amino acids, termed gating charges. These charges move in response to depolarization, resulting in the partial outward translocation of the S4 segment through the membrane. The S5 and S6 segments—which flank the pore-forming P-loop—form the actual gating machinery of the channel (Fig. 1a orange segments). The P-loops from all four domains come together and line the pore of the channel. The movement of the S4 segment is transmitted to the neighboring S5 and possible S6 segments (Catterall 2010; Payandeh et al. 2011; Yarov-Yarovoy et al. 2006), resulting in a conformational change that opens the gate. In potassium channels, which form homo- or hetero-tetramers of six transmembrane subunits, the combination of different subunits is a source of high functional heterogeneity. In contrast, the four repeats of the voltage-gated calcium channel are linked together and show considerable structural heterogeneity. While the S4 segments are well conserved between the four repeats, the other transmembrane segments and especially the linkers between them differ greatly both in lengths and amino-acid sequence (see Fig. 1b, Sequence alignment of the S3–S4 linker together with S4 of $Ca_V1.1$ fourth repeat) Therefore, it is likely that the four repeats differentially contribute to the gating of the calcium channel.

In fact, a striking example of the heterogeneous contribution of the four repeats to channel gating comes from the skeletal muscle $Ca_V1.1a$ calcium channel, which activates two distinct voltage-dependent processes. As stated above, $Ca_V1.1a$ rapidly activates EC-

coupling at 30 mV lower voltages compared to gating of the channel itself. Moreover, compared to ECC coupling and to the activation kinetics of other voltage-gated calcium channels $Ca_V1.1a$ currents activate very slowly. In the early 1990s several studies elucidated the mechanism and the molecular determinants of $Ca_V1.1$ specific gating properties. Using skeletal $Ca_V1.1a$ and cardiac $Ca_V1.2$ chimeras two consecutive studies from the Beam laboratory proposed that the first repeat, in particular the transmembrane segment IS3 together with IS3–IS4 linker represent the molecular entity responsible for the specific gating behavior of $Ca_V1.1a$ and $Ca_V1.2$ calcium channels (Tanabe et al. 1991; Nakai et al. 1994). Earlier (Feldmeyer et al. 1990) elegantly showed that in frog muscle the $Ca_V1.1a$ slow activation kinetics can be accelerated using a depolarization pre-pulse. Although, this experiment could never be reproduced in mammalian muscle, these studies suggested an attractive mechanistic model explaining the peculiar gating behavior of the skeletal muscle calcium channel (Melzer et al. 1995). According to this model up to three of the repeats undergo a fast voltage-dependent transition that is sufficient to gate the opening of the RyR1, but not the opening of the $Ca_V1.1a$ channel pore. As it has been demonstrated that the loop connecting repeats II and III represents the link between $Ca_V1.1$ α_1 subunit and RyR1 (Grabner et al. 1999; Kugler et al. 2004; Tanabe et al. 1990), it is reasonable to assume that a conformational change of the voltage sensors in the adjacent repeats II and III, or perhaps only one of them, may be sufficient to open the RyR1 (see Fig. 1). The gating of the channel pore requires the translocation of all four voltage sensors through the membrane. In the classical $Ca_V1.1a$ α_1 subunit this transition is slow and appears to depend on higher voltages than those of the other repeats. According to the work by Nakai et al. (1996) the slowly responding S4 is located in the first repeat. However, the same study indicates that the slow activation kinetics of repeat I is not necessarily coupled to the poor voltage sensitivity of the transition limiting channel opening.

Recently we identified a new splice variant of the skeletal muscle $Ca_V1.1$ calcium channel, which shed light on the molecular entity responsible for the poor voltage sensitivity and small amplitude of $Ca_V1.1$ (Tuluc et al. 2009). This new splice variant, lacks exon 29 which encodes for 19 amino-acids in the extracellular loop between S3 and S4 in the fourth repeat. The $Ca_V1.1e$ splice variant has an eightfold higher current amplitude compared to the full lengths $Ca_V1.1a$ due to an increased open probability and better voltage sensitivity. Very important for the issue at hand is the fact that its voltage dependence of activation is shifted by 30 mV to the left, while the voltage dependence of EC-coupling (when measured in the absence of calcium influx) was not affected. Because in this splice variant lacking exon 29 EC-coupling and calcium currents activate at the same potential, we concluded that in $Ca_V1.1e$ the S4 voltage sensors from all four domains respond equally to the membrane depolarization.

Therefore, the fourth domain and especially the IVS3–IVS4 linker containing exon 29 is the molecular determinant of the specific voltage dependence and amplitude (Tuluc et al. 2009), whereas the first domain (IS3 + IS3–IS4 linker) is mainly responsible for the kinetic properties (Nakai et al. 1994). Apparently, these two voltage-sensors differentially determine the characteristic gating behavior (slow activation kinetics and poor voltage sensitivity, respectively) of the skeletal muscle calcium channel. Because ECC couples fast and at low voltages, it is unlikely that the full conformational transition of either one of these voltage-

sensors is required for activating the RyR1. The exact mechanism how these structures modulate $Ca_V1.1$ calcium current properties and, whether repeats II and III are sufficient for EC-coupling need to be further investigated.

Auxiliary channel subunits as modulator of L-type calcium currents properties

The voltage gated calcium channel is a complex formed by multiple subunits. Besides the major voltage-sensing and pore-forming α_{1S} subunit, skeletal muscle calcium channels contain three auxiliary subunits (Catterall 2000); the extracellular $\alpha_2\delta$-1 (Davies et al. 2010), the intracellular β_{1a}, and the integral membrane γ-1 subunit. The role of the calcium channel subunits has been extensively explored using either heterologous or homologous expression systems and knock-out mouse models (Striessnig and Koschak 2008).

By heterologous coexpression of calcium channel subunits in Xenopus oocytes and mammalian cells it has been shown that the $\alpha_2\delta$-1 subunit is an important determinant of membrane incorporation and calcium current properties. In contrast, in skeletal myotubes we could show that the $\alpha_2\delta$-1 subunit has no role in membrane incorporation or triad targeting of either the skeletal $Ca_V1.1$ or the cardiac $Ca_V1.2$ calcium channels. Instead in muscle cells it functions primarily as an important modulator of the current properties (Flucher et al. 2005; Obermair et al. 2005, 2008; Tuluc et al. 2007). In dysgenic myotubes reconstituted with the endogenous $Ca_V1.1a$, the knock-down of $\alpha_2\delta$-1 subunit using shRNA resulted in calcium currents with altered kinetic properties, while the voltage dependence and amplitude were not affected. The lack of $\alpha_2\delta$-1 subunit strongly accelerated the activation and inactivation kinetics compared to the very slow activating and inactivating currents of dysgenic myotubes expressing the full $Ca_V1.1a$ channel complex (Obermair et al. 2005). Even though the $Ca_V1.1e$ splice variant intrinsically has faster activation and inactivation kinetics, the depletion of $\alpha_2\delta$-1 subunit further accelerated its gating properties (Tuluc et al. 2009), indicating that it is modulated by the $\alpha_2\delta$-1 subunit in essentially the same way as the full-length $Ca_V1.1a$. Interestingly, in dysgenic myotubes reconstituted with cardiac $Ca_V1.2$ the depletion of $\alpha_2\delta$-1 also altered the calcium current kinetic properties but in the opposite direction (Tuluc et al. 2007). Whereas the $Ca_V1.2$ calcium channel complex containing $\alpha_2\delta$-1 has fast activation kinetics, the lack of $\alpha_2\delta$-1 reduced both the activation and the inactivation rates. Computer modeling of cardiac myocytes demonstrated that particularly the delayed inactivation prolongs the cardiac action potential similarly to long QT syndromes and Timothy disease (Splawski et al. 2004, 2005; Tuluc et al. 2007). The lack of $\alpha_2\delta$-1 subunit also shifted the voltage dependence of activation towards more positive potentials and reduced the calcium current amplitude. These results were fully confirmed in vivo in a $\alpha_2\delta$-1 knockout mouse model (Fuller-Bicer et al. 2009). The genetic ablation of $\alpha_2\delta$-1 subunit resulted in cardiac $Ca_V1.2$ calcium currents with slower activation and inactivation kinetics, shifted voltage dependence of activation towards more positive potentials and reduced L-type calcium currents amplitude. The changes in $Ca_V1.2$ calcium current properties resulted in decreased basal myocardial contractibility and relaxation. Thus, the function of $\alpha_2\delta$-1 in muscle cells is to stabilize the kinetic properties intrinsically determined by the α_1 subunit; $\alpha_2\delta$-1 makes the skeletal currents slow and the cardiac

currents fast. Importantly, its action on the α_1 subunit is independent of the presence of exon 29 in the voltage sensor of the fourth repeat.

The β subunits are necessary for the efficient expression of functional channels in the membrane. In addition, the β_4 subunit seems to have functions independent of calcium channel complex (Subramanyam et al. 2009). The over-expression of β_1–β_4 subunits in adult rat cardiac myocytes increased the amplitude of the gating charge currents and L-type calcium currents by augmenting the number of channels functionally incorporated in the membrane. Furthermore, the different β subunits differentially altered the $Ca_V1.2$ inactivation kinetics (Colecraft et al. 2002). In skeletal muscle β_{1a} is the only β subunit expressed and the genetic ablation of β_1 subunit reduces substantially the number of channels expressed in the membrane and completely abolishes the gating charge currents (Gregg et al. 1996; Schredelseker et al. 2005). Interestingly, reconstitution of β_1-null skeletal myotubes with different β isoforms showed that only β_{1a} is capable of restoring the proper interaction and structural organization of the $Ca_V1.1$ and RyR1 and therefore proper skeletal muscle EC-coupling (Schredelseker et al. 2009; Schuhmeier et al. 2005; Sheridan et al. 2004). The β_3 subunit fails to promote membrane incorporation, whereas the β_2 subunit (Sheridan et al. 2004) is capable of promoting $Ca_V1.1$ membrane incorporation but fails to restore the characteristic skeletal muscle EC-coupling. In contrast to heterologous cells it seems that in skeletal muscle β subunits do not affect calcium current voltage dependence or kinetic properties (Sheridan et al. 2004). Thus, the central role of β subunits in skeletal muscle calcium channel complex is promotion of membrane expression, with an exclusive role of β_{1a} in coupling of $Ca_V1.1$ to the RyR1.

So far only skeletal muscle calcium channel complexes have been demonstrated to contain a γ subunit, and other γ isoforms (stargazing) have been shown to be associated with glutamate receptors in the brain (Kato et al. 2007, 2008; Vandenberghe et al. 2005). In heterologous expression systems, coexpression of the skeletal muscle $\gamma1$ subunit enhances the activation and inactivation kinetics, and shifts the voltage dependence of inactivation of cardiac $Ca_V1.2$ calcium channel (Singer et al. 1991; Sipos et al. 2000). However, this $\gamma1$ subunit is exclusively expressed in skeletal muscle and therefore the physiological role of $\gamma1$ subunit could be tested only in $\gamma1$-null mice (Ahern et al. 2001; Freise et al. 2000). Functional analysis of $\gamma1$-null myotubes showed that $\gamma1$ subunit accelerates the activation and inactivation kinetics of $Ca_V1.1$ calcium currents (Ahern et al. 2001; Ursu et al. 2001). Interestingly, in $\gamma1$-null muscle fibers, the $Ca_V1.1$ calcium current kinetics are similar to calcium currents recorded from control fibers (Ursu et al. 2004). But, the $\gamma1$ subunit shifts the voltage dependence of calcium current inactivation and voltage dependence of inactivation of calcium release from RyR towards more depolarizing potentials in both myotubes and fibers (Ursu et al. 2004). Therefore the $\gamma1$ subunit has been suggested to act as an endogenous $Ca_V1.1$ calcium channel antagonist (Andronache et al. 2007) and, in concert with the $\alpha_2\delta$-1 subunit (Obermair et al. 2005), limits the L-type calcium current in skeletal muscle, although by a different mechanism.

Together this evidence shows that in skeletal muscle auxiliary calcium channel subunits are capable of modifying channel targeting (β_{1a}) and the calcium channel gating and current properties ($\alpha_2\delta$-1, $\gamma1$). However, the mechanisms by which they exert their effect on the

Ca_V α_1 subunit is largely unknown. So far only the interaction site between the β subunits and the α_1 subunit has been characterized (Chen et al. 2004; De Waard et al. 1996; Pragnell et al. 1994). In contrast there is comparably little evidence for the molecular mechanism responsible for β subunits modulatory effects (Barrett and Tsien 2008). As stated above in skeletal muscle the strongest modulator of $Ca_V1.1$ calcium current properties is the $\alpha_2\delta$-1 subunit. Attempts have been made to identify the interaction site between the Ca_V α_1 subunit and the $\alpha_2\delta$-1 subunit (Felix et al. 1997; Gurnett et al. 1997) but so far the exact sequence involved in this interaction and the modulatory mechanism are still elusive. Consistent with its extracellular localization, the $\alpha_2\delta$-1 subunit exerts its modulatory functions extracellularly, possibly via single or multiple interactions. The extracellular loops between IS3-S4 and IVS3-S4 are critical determinants of calcium current kinetics and voltage dependence, respectively. As the $Ca_V1.1e$ isoform is still modulated by $\alpha_2\delta$-1, we ruled out the possibility that the $\alpha_2\delta$-1 subunit interacts with $Ca_V1.1$ α_1 subunit at the IVS3-S4 linker (Tuluc et al. 2009). Interestingly, the lack of the $\alpha_2\delta$-1 subunit (Gach et al. 2008; Obermair et al. 2005) results in $Ca_V1.1$ calcium currents with fast activation and inactivation kinetics, with characteristics identical to calcium currents recorded earlier from chimeric channels in which the first repeat had been replaced with the corresponding domain from the cardiac $Ca_V1.2$ calcium channel (Tanabe et al. 1991). Future research needs to examine whether this is the amino acid sequence through which $\alpha_2\delta$-1 subunit exerts its modulatory function.

Physiological role of L-type calcium currents in skeletal muscle

The contribution of L-type calcium currents to skeletal muscle EC coupling is negligible and therapeutic use of L-channel blockers does not affect mature skeletal muscle function (Melzer et al. 1995). Therefore L-type calcium currents were never considered to be of substantial importance for mature muscle function and the question as to the role of L-type calcium currents in mammalian skeletal muscle functions remained unanswered. Nevertheless, the growing number of channelopathies associated with $Ca_V1.1$ (Jurkat-Rott and Lehmann-Horn 2005; Jurkat-Rott et al. 2000; Pirone et al. 2010; Striessnig et al. 2010) indicates that the L-type calcium currents are essential for muscle function, but the mechanism how the $Ca_V1.1$ calcium channel mutations affect the skeletal muscle EC-coupling is still elusive. In fact, considering that during the brief depolarization of an action potential the channel may not open at all, it is difficult to conceive how altered current properties should lead to muscle defects. However, the short splice variant lacking exon 29 has a very high current amplitude and in myotubes expressing $Ca_V1.1e$ EC-coupling has a cardiac-like calcium-dependent calcium release component (Tuluc et al. 2009). The sizeable calcium currents through $Ca_V1.1e$ contribute directly to the myoplasmic calcium concentration and possibly also indirectly by augmenting RyR1 calcium release via calcium-induced calcium release. Whereas adult muscles express very small amounts of the short splice variant, in developing myotubes $Ca_V1.1e$ is the dominant splice variant (Flucher and Tuluc 2011). One possibility how this channel might nevertheless contribute to the development of progressive myopathies were, if morphological defects of the myofibers went hand in hand with a shift in expression patterns from adult to embryonic splice variants. Ectopic expression of $Ca_V1.1e$ in adult fibers would be expected to lead to calcium

overload and thus might activate disease pathways or cause cell damage. Future research needs to determine whether $Ca_V1.1e$ is up-regulated in diseased muscles or in myopathy animal models and whether block of L-type calcium currents counteracts the disease progression.

Role of L-type calcium currents in excitation-coupled calcium entry

Entry of divalent ions into skeletal muscle in response to sustained depolarization or repetitive stimulation has been termed excitation-coupled calcium entry (ECCE) (Cherednichenko et al. 2004). ECCE is a phenomenon which may be important for maintaining force generation during tetanic stimulation and may contribute to the pathologically increased calcium concentration during episodes of malignant hyperthermia (Cherednichenko et al. 2008; Yang et al. 2007). ECCE requires both the $Ca_V1.1$ and the RyR1, but initially was thought to be distinct of the L-type calcium current. Instead, based on its pharmacological profile, transient receptor potential channels or store-operated calcium entry have been suggested to be the basis of ECCE (Cherednichenko et al. 2008; Hurne et al. 2005; Lyfenko and Dirksen 2008). Most recent evidence indicates that the skeletal L-type calcium current is the major contributor to ECCE (Bannister et al. 2009). Actually ECCE can also be reconstituted in dysgenic ($Ca_V1.1$-null) myotubes by expression of the cardiac $Ca_V1.2$ (Bannister and Beam 2009). This channel isoform has current properties similar to those of $Ca_V1.1e$. Whether the adult $Ca_V1.1a$ isoform is also capable of reconstituting ECCE in dysgenic muscle has not been addressed. Furthermore, the depletion of the auxiliary $\alpha_2\delta$-1 calcium channel subunit resulted in a complete loss of ECCE during KCl depolarization and a more rapid decay of calcium transients during bouts of repetitive electrical stimulation like those occurring during normal muscle activation in vivo (Gach et al. 2008). Thus, ECCE requires the L-type calcium current but not skeletal muscle-type EC coupling. Considering the small amplitude and poor voltage sensitivity of the long form $Ca_V1.1a$, it seems very unlikely that this channel can supply the calcium current required for ECCE. But, ECCE—just like expression of $Ca_V1.1e$—is preferentially found in developing myotubes. Therefore, it is much more likely that the molecular entity responsible for ECCE is the embryonic $Ca_V1.1e$ isoform.

Role of L-type calcium currents in neuromuscular junction formation

In the early 1980s Pinçon-Raymond, Powell, and Rieger published a series of articles (Pincon-Raymond and Rieger 1982; Powell et al. 1984; Rieger et al. 1984) in which they thoroughly describe the neuromuscular junction (NMJ) phenotype in the dysgenic mouse (mdg). Later this mouse was identified as a spontaneous null-mutant of the skeletal muscle $Ca_V1.1$ α_1 subunit (Knudson et al. 1989; Tanabe et al. 1988). One of the earliest steps in the development of the NMJ is the regular arrangement of acetylcholine receptors (AChR) in the center of muscle fibers, termed pre-patterning. Using ^{125}I-α-bungarotoxin autoradiography of AChRs and a range of other techniques the authors observed that compared to normal embryonic muscles, dysgenic diaphragm muscles expressed multiple endplates per fiber and increased amounts of extrajunctional AChR (Flucher and Tuluc 2011). The AChR clusters were not concentrated in a single centrally localized band but broadly dispersed over the muscle. Furthermore they found that this aberrant postsynaptic patterning is correlated with

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increased nerve sprouting, extended innervations territories and ultrastructurally immature features of the NMJs. Recently, the developmental defects in AChR clustering and innervation patterns were found in another calcium channel knock-out mouse model (Chen et al. 2011). The distribution of AChR and branching of the phrenic nerve were studies in diaphragms of embryos of the calcium channel β_1 (*Cacnb1*$^{-/-}$) knock-out mouse earlier generated by (Gregg et al. 1996). The lack of β_1 subunit results in a reduced number of channels functionally incorporated in the membrane and complete loss of L-type calcium currents and EC-coupling. Therefore, the *Cacnb1*$^{-/-}$ mouse and the dysgenic (*Cacna1S*$^{-/-}$) mouse models are functionally equivalent in that both lack L-type calcium currents and EC-coupling; consequently they both die at birth from respiratory failure.

$Ca_V1.1a$ L-type calcium currents have such a small current amplitude and poor voltage-sensitivity that during 200 ms depolarizations in combined voltage-clamp and calcium imaging experiments their contribution to the myoplasmic calcium transient is not detectible (Flucher and Tuluc 2011; Schuhmeier et al. 2005). During the brief depolarization of a single action potential or during spontaneous electrical activity in developing muscle fibers prior to innervation, it is very unlikely that the pores of these channels open at all. Thus, for all we know about the classical skeletal muscle calcium channel, $Ca_V1.1a$ cannot be the source of the L-type calcium currents activating NMJ pre-patterning. However, the $Ca_V1.1e$ splice variant lacking exon 29 has a large current amplitude and left-shifted voltage dependence of activation. In contrast to the full-length $Ca_V1.1a$ calcium entering through $Ca_V1.1e$ during 200 ms step depolarization substantially contributes to cytosolic calcium concentration. Furthermore, $Ca_V1.1e$ is the dominant splice variant (~80%) in both mouse and human myotubes (Tuluc et al. 2009). Therefore the expression pattern and the biophysical properties of $Ca_V1.1e$ implicate it in the neuromuscular junction formation during embryonic development (Flucher and Tuluc 2011).

Conclusion

For many years it has been accepted that the sole role of the skeletal muscle calcium channels $Ca_V1.1$ is to trigger the opening of the sarcoplasmic reticulum calcium release channel RYR1 and thus trigger skeletal muscle EC-contraction. Yet, there is substantial new evidence that implicates the skeletal muscle calcium channel in processes that depend on the influx of calcium through $Ca_V1.1$; most prominently ECCE and the formation of the neuromuscular junction. To date we know two splice variants of the $Ca_V1.1$ α_{1S} subunit expressed in skeletal muscle. In the classical long isoform ($Ca_V1.1a$) the auxiliary $\alpha_2\delta$-1 and γ1 subunits as well as unique intrinsic structures in the voltage-sensor of the first and fourth repeat (the latter including exon 29) limit the kinetics and voltage-sensitivity of the calcium current. In contrast, the newly discovered short splice variant ($Ca_V1.1e$), which lacks exon 29 and has a high current amplitude, fast activation kinetics and normal voltage sensitivity, is a very efficient calcium channel. The expression of such dramatically different channel variants indicate fundamentally different functions in developing and mature skeletal muscle, respectively. While both splice variants support the direct, calcium independent coupling of the voltage sensor and RyR1, only the embryonic $Ca_V1.1e$ variant functions as genuine L-type calcium channel and may thus be of physiological and clinical importance.

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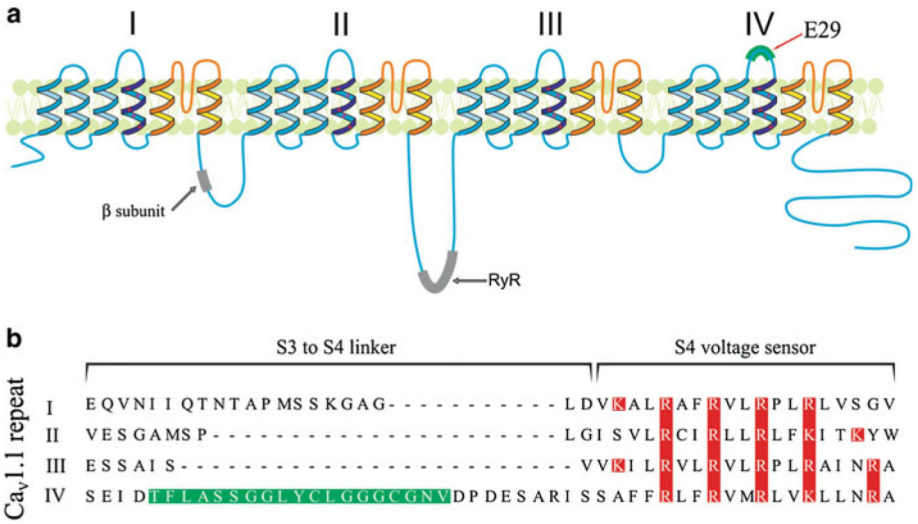

Fig. 1.

Structure of the skeletal muscle calcium channel α_{1S} subunit. **a** Predicted transmembrane topology of $Ca_V1.1$ α_{1S} subunit. The regions of the cytoplasmic loops interacting with the β subunit and the RyR1 are emphasized in *gray*, while the location of exon 29, which is missing in the $Ca_V1.1e$ isoform is highlighted in *green*. **b** Sequence alignment of the extracellular S3–S4 linkers together with the S4 transmembrane segment from repeats one to four of the skeletal muscle calcium channel. The amino acid sequence encoded by the exon 29 in IVS3–S4 linker is highlighted in *green*, while *red* letters emphasize the positively charged residues responsible for the detection of the membrane depolarization. (Color figure online)