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$Ca_V1.1$: The atypical prototypical voltage-gated Ca^{2+} channel

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Abstract

$Ca_V1.1$ is the prototype for the other nine known Ca_V channel isoforms, yet it has functional properties that make it truly atypical of this group. Specifically, $Ca_V1.1$ is expressed solely in skeletal muscle where it serves multiple purposes; it is the voltage sensor for excitation-contraction (EC) coupling and it is an L-type Ca^{2+} channel which contributes to a form of activity-dependent Ca^{2+} entry that has been termed Excitation-Coupled Ca^{2+} Entry (ECCE). The ability of $Ca_V1.1$ to serve as voltage-sensor for EC coupling appears to be unique amongst Ca_V channels, whereas the physiological role of its more conventional function as a Ca^{2+} channel has been a matter of uncertainty for nearly 50 years. In this chapter, we discuss how $Ca_V1.1$ supports EC coupling, the possible relevance of Ca^{2+} entry through $Ca_V1.1$ and how alterations of $Ca_V1.1$ function can have pathophysiological consequences.

Keywords

Dihydropyridine receptor (DHPR); $Ca_V1.1$; α_{1S}; L-type; excitation-contraction (EC) coupling; Excitation-Coupled Ca^{2+} Entry (ECCE)

$Ca_V1.1$ is the prototypical voltage-gated Ca^{2+} channel. Like the other L-type ($Ca_V1.X$) Ca^{2+} channels, it is defined by its being responsive to inhibition or potentiation by 1,4-dihydropyridines. It was the first of the Ca_V family to be cloned [1] and it was the first ion channel to have a null mouse model [2]. Yet $Ca_V1.1$ is so atypical of the Ca_V family, one could say that it is the unicorn of the family. Compared to the other nine Ca_V channels, it activates slowly and inactivates even more slowly. Its expression is restricted to one tissue—skeletal muscle—where it is the only Ca_V channel expressed in differentiated fibers. Moreover, $Ca_V1.1$ requires the influence of the type 1 ryanodine receptor (RyR1) to support its L-type channel function. However, the physiological significance of L-type Ca^{2+} current via $Ca_V1.1$ is uncertain [3,4], and the ability of $Ca_V1.1$ to carry out its most important function does not depend on Ca^{2+} flux at all [5,6].

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$Ca_V1.1$ is the prototypical Ca_V channel

As noted above, α_{1S} ($Ca_V1.1$) was the first principal α_1 subunit of a Ca_V channel for which a complete cDNA sequence was obtained, which like the initial cloning of many other ion channels was accomplished by the Numa laboratory in the 1980's [1]. Their work made use of rabbit skeletal muscle, which abundantly expresses receptors for 1,4-dihydropyridine receptors (DHPRs), although it must be noted that the equivalence of DHPRs and voltage-gated Ca^{2+} channels was at the time a source of much debate [7,8] In any event, sequencing of tryptic fragments of purified DHPRs was used to design oligonucleotide primers for reverse transcription of muscle mRNA. From the resulting cDNA sequence, they concluded that the basic structure was much like the first rat brain Na^+ channel (now known as $Na_V1.1$) which they had cloned just prior [9]. Like $Na_V1.1$, the α_{1S} subunit of the channel had four repeats, having sequence similarity with one another (Fig. 1). The amino- and carboxyl-termini, as well as the loops linking the four repeats (I-II, II-III, III-IV) were proposed to be cytoplasmic. These cytoplasmic regions are now known to be important for interactions with other proteins and play roles in gating, expression and targeting of $Ca_V1.1$ [reviewed in 10]. Each of the four repeats is comprised of six α-helices, and the fourth helix (S4) of each repeat has a distinctive motif of evenly spaced basic residues which correspond to the voltage-sensing particle that Hodgkin and Huxley had proposed thirty years earlier for neuronal Na^+ channels [11]. Importantly, the channel differed from $Na_V1.1$ in that the residues that corresponded to the Na^+ channels' D-E-K-A selectivity filter were E-E-E-E [12,13]. This tetra-acidic structure was postulated to facilitate selective passage of Ca^{2+} and other divalent cations such as Ba^{2+}, Mg^{2+}, Mn^{2+} and Sr^{2+} through the pore of L-type channels by providing binding sites for two separate divalents [14]. To this day, this motif is known to be important for the selectivity of $Ca_V1.1$; neutralization of just one of these residues in the third conserved repeat will nearly ablate the ability of the channel to conduct divalents and will convert it into a Na^+-selective channel [5,15-17].

$Ca_V1.1$ is assembled as heteromultimer together with $\alpha_2\delta$-1, β_{1a} and γ_1 subunits

The skeletal muscle L-type Ca^{2+} channel is comprised of $Ca_V1.1$ and auxiliary $\alpha_2\delta$-1, β_{1a}, and γ_1 subunits. With the exception of $\alpha_2\delta$-1, expression of each of these subunit isoforms is largely restricted to skeletal muscle [18,19, but see 20]. Ablation of either $\alpha_2\delta$-1 or γ_1 expression has only modest effects on channel current density [21-26]; the main effect of $\alpha_2\delta$-1 is to slow activation kinetics [24-26] and that of γ_1 is to cause a depolarizing shift of channel inactivation [21-23], but the broader physiological significance of these effects is not clear. On the other hand, β_{1a} is nearly essential for channel function [27,28]. Like $Ca_V\beta$ subunit isoforms in other tissues, the β_{1a} subunit facilitates trafficking of $Ca_V1.1$ to the plasma membrane. However, the specific targeting of the assembled $Ca_V1.1$ channel complex to the junctional membrane is dependent on $Ca_V1.1$ [29-31]. Within the junctional membrane, individual $Ca_V1.1$-containing heteromultimers are arranged into groups of four ("tetrads"), in which the four $Ca_V1.1$ channels are aligned with the four subunits of every other RyR1 homotetramer (Fig. 2-*left*) [30, 32-38]. By itself, the highly-registered tetradic organization of $Ca_V1.1$ at triad junctions is perhaps the most concrete evidence of physical interactions linking $Ca_V1.1$ to RyR1. This view is fortified by the observations that long duration pharmacological manipulation of RyR1 conformation with high concentrations of ryanodine (>200 μM) reduces the average, center-to-center distance between adjacent DHPRs within a tetrad [38] and shifts activation of both L-type current [39,40] charge movement [40] to more hyperpolarized test potentials.

In addition to promoting trafficking of the $Ca_V1.1$ complex to the membrane, β_{1a} is essential for efficient tetrad formation. Triad junctions of the β_1-null zebrafish mutant *relaxed* display

randomly positioned $Ca_V1.1$ particles in freeze-fracture replicas (Fig. 2-*right*); transgenic overexpression of β_{1a} in *relaxed* embryos restores the orthogonal $Ca_V1.1$ tetradic arrays [30]. The rescue of tetrads is an exclusive function of β_{1a} [37]. The observations made in the zebrafish model system make it tempting to envision β_{1a} as the molecular glue that holds tetrads together by linking $Ca_V1.1$ to RyR1. Interestingly, the possibility of a direct interaction of β_{1a} with a cluster of positively charged amino acids (3495-3502) in the cytoplasmic amino-terminal region of RyR1 has been raised based on the *in vitro* binding of β_{1a} to a another fragment of RyR1 (3490-3523) inclusive of this stretch of residues [41]. In more recent studies, peptides corresponding to a minimal sequence (residues 490-524) within the most distal 35 residues of the β_{1a} carboxyl-terminus were found to bind to native RyR1 in SR vesicles [42,43]. In contrast to these *in vitro* data which suggest that β_{1a} can bind to RyR1 independently of $Ca_V1.1$, β_{1a} does not appear to bind to RyR1 *in vivo* in $Ca_V1.1$-null (*dysgenic*) myotubes [29, 31,44]. These latter data imply that if an association between β_{1a} and RyR1 occurs *in vivo*, this association requires the presence $Ca_V1.1$.

$Ca_V1.1$ is the voltage sensor for EC coupling

Excitation-contraction (EC) coupling in skeletal muscle requires the transduction of a plasmalemmal depolarization within the transverse-tubule network into a transient elevation in myoplasmic Ca^{2+} emanating from the stores of the sarcoplasmic reticulum (SR) that activates the contractile filaments [45]. It has been known since the early 1970's that EC coupling in skeletal muscle does not require the entry of extracellular Ca^{2+} [46] but relies on the outward movement of charged, membrane-bound gating particles [47]. The identity of these voltage-sensing particles would remain a mystery for another decade, at which point it was observed that myotubes cultured from a perinatally lethal mouse line (*dysgenic*) lacked dihydropyridine-sensitive voltage-gated Ca^{2+} current (Fig. 3-*middle column*) [2]. Around the same time, Ríos and Brum [48] demonstrated that charge movement and EC coupling were inhibited in parallel by the dihydropyridine antagonist nifedipine. Together, these independent works fingered the L-type Ca^{2+} channel/DHPR as the voltage sensor. Nearly absolute proof came along a short time later when the newly cloned $Ca_V1.1$ restored L-type current, charge movement and EC coupling when expressed in *dysgenic* myotubes (Fig. 3-*right column*) [49-52] and today $Ca_V1.1$ is universally believed to be the voltage-sensor for EC coupling in skeletal muscle.

A recent *in vivo* example underscores the lack of a requirement for Ca^{2+} entry via $Ca_V1.1$ for skeletal-type EC coupling. Zebrafish have two distinct $Ca_V1.1$ α_{1S} subunit isoforms (α_{1S}-a and α_{1S}-b) that have both lost the ability over time to conduct L-type current [53]. These two channels have distinct residue substitutions in the pore helix that block Ca^{2+} permeation while sparing the ability of both to trigger EC coupling. Specifically, α_{1S}-a has a tryptophan-glycine doublet in place of an methionine-glutamate pair in the P-loop of Repeat I and α_{1S}-b has an aspartate for asparagine swap near the selectivity filter of Repeat II. These non-conducting channels are characteristic of higher teleosts, but the evolutionary pressure driving the non-conducting amino acid substitutions in zebrafish and other bony fish [53] remains a mystery.

The non-channel function of the EC coupling voltage-sensor makes $Ca_V1.1$ truly peculiar amongst Ca_V channels since it is the only isoform capable of efficiently coupling membrane depolarization to activation of an RyR via a Ca^{2+} entry-independent, conformational coupling mechanism [51,54,55]. The only other similar phenomenon that has been described is intracellular Ca^{2+} release in nerve terminals of the neurohypophysis, which depends upon an interaction between a yet-to-be determined $Ca_V1.X$ channel and RyR1 [56-58]. In any event, the fairly well-conserved $Ca_V1.2$ channel cannot gate RyR1 in cultured *dysgenic* myotubes without Ca^{2+} flowing into the myoplasm through the channel pore [5,54,55]. The

inability of $Ca_V1.2$ [51,54,59], $Ca_V2.1$ [60], $Ca_V3.2$ [61] and the *Musca domesticus* muscle L-type channel [62,63] to restore conformational coupling with RyR1 in *dysgenic* myotubes enabled the investigation of key elements for EC coupling within $Ca_V1.1$ via chimeric approaches. The studies that utilized $Ca_V1.1$-based chimeras arrived at the conclusion that II-III linker was a structure indispensable for skeletal-type EC coupling [51] and, in particular, the sequence spanning residues 720-764/5 in the center of the linker were "critical" for this function (Fig. 1) [59,62,64]. Kugler and colleagues suggested that residues 744-751 within the critical domain form a random coil conformation (rather than an α-helix), and that this random coil enables $Ca_V1.1$ to interact with other junctional proteins (e.g., RyR1) upon membrane depolarization [64]. In this study, Kugler et al. also identified four amino acids within the critical domain (A739, F741, P742, D744) which they suggested deter formation of the α-helical conformation of the II-III loop that impedes EC coupling.

The carboxyl-terminal portion of the loop connecting the "critical domain" to Repeat III, a region which displays substantial sequence similarity amongst L-type Ca^{2+} channels, was later identified as a key structure in a study that employed insertion of fluorescent protein (Fig. 1) [65]. $Ca_V1.1$ channels which had YFP introduced at either residue 760 or 785 were incapable of supporting voltage-dependent Ca^{2+} release from the SR despite somewhat normal channel expression and the apparent retention of the structural integrity of the critical domain. At this point, it is not known whether the carboxyl-terminal portion of the II-III linker is directly involved in gating RyR1 during EC coupling or whether it is merely a specialized conduit for communication between the critical domain and the channel's voltage-sensing elements.

Although there is general consensus that the II-III loop is an essential player in skeletal-type EC coupling, a handful of studies that have applied synthetic peptide mimics of II-III loop segments to isolated RyR1 in reconstituted lipid bilayers have produced results which led to the notion that a segment of the amino-terminal portion of the II-III loop known as the "A domain" (residues 681-690, minimally; Fig. 1) directly interacts with, and activates, isolated RyR1 [66-68]. Most recently, an A domain peptide was found to bind an isolated segment of RyR1, the ubiquitous SPRY2 domain, and this interaction supported increased P_o of RyR1 in lipid bilayers [69,70]. While provocative, experiments that have tested the importance of the A domain for EC coupling in a cellular context have failed time and time again. Specifically, scrambling or total ablation of this region has little or no effect on the magnitude or voltage-dependence of SR Ca^{2+} release in response to depolarization [44,65,71-76].

Roles for the other intracellular linkers in EC coupling and/or other general $Ca_V1.1$ functions have also been identified through deletion or chimera strategies. The $Ca_V1.1$ I-II loop is the site for interaction with the β_{1a} subunit, which, as discussed above, supports membrane trafficking and is required for the tetradic arrangement of $Ca_V1.1$ within triad junctions (Fig. 2-*right*). Skeletal-type EC coupling is absent in muscle cells genetically null for β_{1a} [27,28,30] and is restored by expression of β_{1a} and, to a much lesser extent by β_{2a} [37,77], whereas β_3 and β_4[78] and the *Musca domesticus* β_M [37] are all ineffective.

Based initially on the analysis of deletion mutants and of β_{1a}/β_{2a} chimeras [77,79], much current attention is focused on the role of the carboxyl-terminus of β_{1a} in EC coupling. A synthetic peptide corresponding to the carboxyl-terminal 35 residues of β_{1a} binds to purified RyR1s *in vitro* and, much like A domain peptides, activates RyR1s in lipid bilayers [42]. Within this stretch of residues, a hydrophobic heptad motif unique to β_{1a} was identified as being an important element for EC coupling and triple mutation of three of these residues (L478, V485 and V492) to alanine impaired the ability of rabbit β_{1a} to support EC coupling in mouse β_1 null myotubes [80]. However, the same mutations were inconsequential to EC

coupling and tetrad formation when expressed in zebrafish *relaxed* (β_1 null) myotubes [81] and β_{1a}-based peptides with these substitutions enhanced RyR1 P_o to the same extent as peptides with wild-type sequence [43]. Though the requirement for the presence of the β_{1a} carboxyl-terminus for the EC coupling is widely accepted, these diametrically opposed results obtained in divergent (mammalian *vs.* osteoicthyes and lipid bilayers) systems have given rise to uncertainty of the role of the hydrophobic heptad repeat in coupling with RyR1.

The influences of the other intracellular regions of $Ca_V1.1$ on EC coupling are not as prominent as that of the I-II and II-III loops. The short amino-terminus appears largely inert [82]. The III-IV loop is not directly involved in EC coupling but indirectly can control Ca^{2+} release from the SR by influencing $Ca_V1.1$ gating [83]. The III-IV loop also is the only intracellular site on $Ca_V1.1$ for mutations linked to the pharmacogenetic disorder malignant hyperthermia [84-86]. Finally, the carboxyl-terminus plays a part in channel expression and targeting of the channel to triad junctions [60,87] and is the site for many intermolecular interactions [reviewed in 10].

$Ca_V1.1$ channel properties are atypically dependent on conformational coupling with RyR1

The unique conformational coupling mechanism between $Ca_V1.1$ and RyR1 is bidirectional. As described above, the EC coupling, or “orthograde,” signal is transduced from the voltage-sensing regions of $Ca_V1.1$ to the pore region of RyR1 via the cytoplasmic foot region of RyR1 (Fig. 4). The first recordings of L-type currents from *dyspedic* (RyR1 null) myotubes revealed that there is also an RyR1-mediated “retrograde” signal that is communicated to the channel-activating machinery of $Ca_V1.1$. In particular, very low amplitude L-type currents were observed in *dyspedic* myotubes by Nakai and colleagues [88], despite nearly normal membrane expression of $Ca_V1.1$ inferred from measurement of charge movements. The reduction in current density was corrected by exogenous expression of RyR1. Thus, the increase in conductance to charge ratio indicated that the presence of RyR1 increases $Ca_V1.1$ currents by elevating channel relative P_o. The retrograde signal influences $Ca_V1.1$ activation kinetics [89-91] and, like orthograde coupling, is dependent on the integrity of the critical domain [92].

Interestingly, RyR1 mutations that alter RyR1 function also affect certain biophysical properties of $Ca_V1.1$. For instance, hyperpolarizing shifts in channel activation have been consistently observed in animal models of malignant hyperthermia caused by RyR1 mutations. Gallant et al. [93] saw a small hyperpolarizing shift in activation of L-type Ca^{2+} current in swine myotubes homozygous for the RyR1 R615C mutation, but speculated that series resistance errors could have been responsible. A nearly 10 mV hyperpolarizing shift in $Ca_V1.1$ activation was also reported for myotubes obtained from engineered Y522S homozygous mice [94], as was a small (~3 mV), but significant, hyperpolarizing shift in adult Y522S heterozygous *interosseus* fibers [95]. Similarly, our group observed about a 7 mV hyperpolarizing shift in L-type current activation for mouse myotubes homozygous for the engineered R163C mutation [96]. Hyperpolarizing shifts in charge movement have also been observed for both R163C myotubes [96] and Y522S fibers [95], but not in swine R165C myotubes [97]. In regard to EC coupling, each of these malignant hyperthermia models [R163C, Y522S, R615C; 94-96,98,99] and *dyspedic* myotubes expressing RyR1 constructs carrying a number of different malignant hyperthermia-linked mutations [100] have also displayed hyperpolarizing shifts in SR Ca^{2+} release in response to depolarization suggesting that RyR1 malignant hyperthermia mutations shift the equilibrium of $Ca_V1.1$ towards the state(s) active for EC coupling and L-type channel activation.

$Ca_V1.1$ displays depolarization-induced shifts into high P_o mode 2 gating

$Ca_V1.1$ and the other L-type channels have three broadly-defined gating modes which have been characterized both at macroscopic and single channel levels [17,101]. Mode 0 represents the closed state of the channel, mode 1 is characterized by brief (~1 ms) openings and mode 2 displays longer duration openings which are promoted both by exposure to 1,4-dihydropyridine agonists (e.g., (−)Bay K 8644) and by strong depolarization [101-104]. On the macroscopic level, the point of entry into mode 2 is difficult to assess during depolarizing step potentials and is most evident in the augmented amplitude and slowed decay of tail currents elicited by repolarization from steps to more negative potentials [17,105].

Does $Ca_V1.1$ undergo calcium-dependent inactivation?

Another means in which $Ca_V1.1$ differs from other high voltage-activated Ca_V channels is its apparent lack of calcium-dependent inactivation (CDI) [106]. This inhibitory feedback mechanism has been rigorously investigated in $Ca_V1.2$, $Ca_V1.3$, and $Ca_V2.1$ channels and been found to require anchoring of calmodulin (CaM) to a conserved IQ motif in the carboxyl-termini of each of these the channels [107-110]. Even during longer depolarizations that certainly facilitate substantial Ca^{2+} entry [15], wild-type $Ca_V1.1$ inactivates very little and what little inactivation there is appears to be dependent on test potential rather than current amplitude. The lack of CDI for native L-type channels in myotubes could be a consequence of factors intrinsic to $Ca_V1.1$. For example, introduction of non-conserved $Ca_V1.1$ residues into and near the IQ domain of $Ca_V1.2$ ablates both CaM-binding to the carboxyl-terminus and CDI in HEK293 cells [111]. Interestingly, $Ca_V1.2$ channels which normally display considerable CDI in both native and heterologous systems [see CDI paper of this series], inactivate very little when expressed in *dysgenic* myotubes [51,54]. This latter observation suggests that extrinsic factors related to the architecture of triad junctions could also contribute to the lack of CDI for $Ca_V1.1$. It must be noted that mild CDI has been reported in cultured normal myotubes [112] and adult mouse *flexor digitorum brevis* (*FDB*) fibers [113]. Thus, the topic of whether $Ca_V1.1$ is prone to CDI merits further investigation in light of these latter results.

Excitation-Coupled Ca^{2+} Entry and $Ca_V1.1$

Over many years, the extremely slow activation of the skeletal muscle L-type Ca^{2+} current has cast doubt on whether $Ca_V1.1$ actually can mediate significant Ca^{2+} entry into muscle fibers during the short duration (~5 ms) of a tubular action potential. The discovery of Excitation-coupled Ca^{2+} Entry (ECCE) by Cherednichencko and colleagues [114] has sparked new interest in this topic. ECCE was initially described as a large, slowly developing mode of Ca^{2+} entry into skeletal muscle that occurs in response to either repetitive or prolonged membrane depolarization [26,114-119]. Such Ca^{2+} entry was detected in both cultured myotubes and adult muscle fibers, either as Ca^{2+} transients in the presence of ryanodine or as Mn^{2+} quench of the ratiometric Ca^{2+} indicator Fura-2 [26,114,115,117,118]. ECCE has also been assessed indirectly in cells in which SR Ca^{2+} release has not been blocked by ryanodine as the difference between the Ca^{2+} transients measured in the presence and absence of external Ca^{2+} [15,116,119]. Interestingly, ECCE was absent in myotubes that are null for either RyR1 or $Ca_V1.1$ α_{1S} or β_{1a} subunits [15,16,114,115,118]. It was also sensitive to block by dihydropyridine antagonists, large di- and trivalent cations and other widely used non-selective cation channel blockers such as SKF-96356 and 2-aminoethyl diphenylborate (2-APB) [15,114,116]. At first glance, the ablation of ECCE in these cases would have pointed directly to the L-type channel as the mediator of ECCE. Even so, Cherednichenko et al. [114] proposed that ECCE is

independent of L-type Ca^{2+} current via the pore of $Ca_V1.1$ because ECCE (as assessed by Mn^{2+} quench of Fura-2 dye) persisted in *dysgenic* myotubes transfected with an $Ca_V1.1$ pore mutant (SkEIIIK) thought to conduct only outward monovalent flux [5]. The persistence of ECCE in SkEIIIK-expressing *dysgenic* myotubes led to the idea that another Ca^{2+}-permeable channel whose gating was coupled directly to the intact $Ca_V1.1$-RyR1 complex was responsible for conducting the Ca^{2+} entry attributed to ECCE.

Initial investigations of the molecular basis of ECCE focused on TRPC3 channels which are abundantly expressed in skeletal muscle [120] and Orai1/STIM1 complexes responsible for store-operated Ca^{2+} entry in skeletal muscle [118,121,122]. For both, ECCE seemed to be unaffected by siRNA or dominant-negative constructs targeting either channel type [118,123]. The lack of contribution from either these channels prompted a re-examination of the $Ca_V1.1$ pore as the ECCE pathway. Indeed, the native L-type channel conducted Mn^{2+} and supported ECCE-like Ca^{2+} entry during long, weak depolarizations. Moreover, ECCE was substantially reduced in *dysgenic* myotubes expressing SkEIIIK in comparison to normal myotubes [15]. Thus, the majority of Ca^{2+} entry attributed to ECCE seemed actually to flow through Ca 1.1. Still, the lack of Ca^{2+} V entry observed in *dysgenic* myotubes expressing SkEIIIK by Bannister et al. [15] contrasted on the surface with the robust Mn^{2+} quench originally observed by Cherednichencko et al. [114]. These results were reconciled by the fact that SkEIIIK does become permeable to Ca^{2+} or Mn^{2+} when it enters mode 2 gating as it would during long, weak depolarization by elevated K^+ or during repetitive electrical stimulation [15,16].

Even though the molecular identity of the permeation pathway remains a matter of some debate [3,4], the voltage-sensor for activation of ECCE is by definition housed in $Ca_V1.1$. For this reason, any physiological impact of ECCE is directly controlled by $Ca_V1.1$. In the original characterization of ECCE, Cherednichencko et al. [114] showed that the rate of Mn^{2+} quench increases with frequency of electrical field stimulation. During continuous application of tetanic stimuli, Ca^{2+} entry with similar pharmacology to ECCE has been shown to maintain myoplasmic Ca^{2+} levels, suggesting that such dihydropyridine-sensitive Ca^{2+} flux may play a role in store replenishment during vigorous activity.

Mutations in $Ca_V1.1$ are directly linked to pathophysiological alterations of muscle

Mutations in $Ca_V1.1$ have been identified as causative for two congenital muscle pathophysiologies, hypokalemic periodic paralysis (HypoKPP) [124,125] and malignant hyperthermia [126,127]. $Ca_V1.1$ was first identified as being a locus for HypoKPP by Ptáček and colleagues in 1994 [128]. A PCR-based investigation revealed multiple probands with mutations resulting in H/G substitutions for R1239, the second basic residue in the voltage-sensing S4 helix of Repeat IV. At least fourteen other HypoKPP mutations of charged residues in the voltage sensing helices of Cav1.1 (R528H, R900H and R1239H; Fig. 1) and the skeletal muscle Na^+ channel ($Na_V1.4$) have been identified since [129-131]. Curiously, the mild effects which these alterations of the putative voltage-sensing structures of $Ca_V1.1$ and $Na_V1.4$ have on either channel's conventional biophysical characteristics could not explain the onset of a HypoKPP episode satisfactorily [131-139]. For example, electrophysiological analysis of myotubes derived from muscle biopsies of affected individuals carrying the $Ca_V1.1$ Repeat II S4 R528H mutation revealed only a small reduction in current density and slowed activation kinetics [135]. Subsequent work on HypoKPP-linked $Na_V1.4$ mutants revealed the mechanism of channel dysfunction in HypoKPP to be the creation of a transmembrane "gating pore" that conducts protons through the channel via a route other than the classic ionic permeation via the central pore [140-142]. The presence of zwitterionic histidines at the second conserved basic residue

position of in $Na_V1.4$ voltage-sensing helices enables passage of protons when the voltage sensors are in the resting position, thereby enabling the passage of these smallest of monovalent cations into the intracellular space. Electrophysiological analysis of mouse *FDB* fibers in which the human orthologue ($Na_V1.4$ R669H) had been introduced genetically demonstrated that resultant inward ω-current conducted by the gating pore is the basis for muscle fiber depolarization when external K^+ is reduced and is therefore sufficient to trigger a HypoKPP episode [143]. It is thought that mutations in $Ca_V1.1$ S4 helices such as R528H also cause HypoKPP episodes by conducting ω-current in the same manner as $Na_V1.4$ mutants [131]. If this is case, the dysfunction of $Ca_V1.1$ that causes HypoKPP episodes is unrelated to its ability to function as either voltage-sensor for EC coupling or as an L-type Ca^{2+} channel.

In addition to the >120 known causative mutations for malignant hyperthermia in RyR1 [144], five mutations have been identified in $Ca_V1.1$ [84-86,145,146]. These five missense mutations encode the following substitutions: 1) R174W, 2) T1354S 2) R1086H, 3) R1086C, and 5) R1086S (Fig. 1). Of these, the R174W, R1086H and T1354S substitutions have been functionally characterized [146-148]. In each case, the mutation had only subtle effects on the ability of $Ca_V1.1$ to trigger Ca^{2+} release from the SR in response to depolarization. In regard to the effects of the mutations on the ability of the channel to conduct L-type Ca^{2+} current, the primary effects of R1086H and T1354S mutations were lowered relative P_o and accelerated channel activation, respectively [146,147]. The impact of the R174W mutation on L-type current was much more severe than R1086H or T1354S, as it abolished channel activation in response to 200 ms depolarizations [148].

Taken together, the minor effects on EC coupling and the varied functional consequences on the L-type current raise many questions regarding the causative role for $Ca_V1.1$ mutations in malignant hyperthermia susceptibility. Recently, it has been proposed that altered resting coupling between mutant $Ca_V1.1$ and RyR1 may underlie malignant hyperthermia susceptibility. The basis for this proposal is that resting myoplasmic Ca^{2+} levels of *dysgenic* myotubes lacking $Ca_V1.1$ are elevated to levels reminiscent of those in muscle of malignant hyperthermia mouse models and in *dyspedic* myotubes expressing mutant RyR1s [149-151]. The increased resting Ca^{2+} levels observed in *dysgenic* myotubes were rectified by expression of $Ca_V1.1$ [151] but persisted when the $Ca_V1.1$ R174W mutant was introduced into these cells instead [148]. Since the R174W mutation had little effect on EC coupling and abolished channel function, it was proposed that altered resting coupling between $Ca_V1.1$ and RyR1 promotes a ryanodine-insensitive, RyR1-mediated SR Ca^{2+} leak pathway [152] that leads to elevated myoplasmic Ca^{2+} levels and hypersensitivity to halogenated anesthetics in $Ca_V1.1$ R174W-expressing *dysgenic* myotubes.

Reduced $Ca_V1.1$ expression contributes to aging-related strength deficits

Muscle weakness in older individuals is partially attributable to depressed muscle excitability---a process termed “EC uncoupling.” Previous studies have shown that EC uncoupling in both human and rodent muscle is characterized by decreased voltage-triggered SR Ca^{2+} release [153,154]. Concurrent reductions in L-type current amplitude and maximal intramembrane charge movement indicate that reduced SR Ca^{2+} release in older mammals is a consequence of reduced number of EC coupling voltage-sensors (i.e., $Ca_V1.1$ channels) resident in the plasma membrane of the transverse tubule network. This idea is further supported by a reduction in radioactive dihydropyridine binding in muscle membrane preparations from older rats [155]. Although a reduction in membrane expression of $Ca_V1.1$ has been established as the basis of age-related EC uncoupling [156], the molecular signals that drive this process remain unclear.

Interestingly, Piétri-Pouxel [157] recently demonstrated that expression of $Ca_V1.1$ is required for maintaining muscle integrity. In this study, mouse hindlimb muscle that had been infected with siRNA directed to $Ca_V1.1$ had gross atrophy, reduced fiber diameter and substantially more fibrosis that the control contralateral muscle. Although the authors of the study did not attempt to determine whether the atrophic effects of $Ca_V1.1$ ablation were a consequence of loss of EC coupling, L-type current, and/or downstream use-dependent contractile activity, their findings raise the possibility that $Ca_V1.1$ activity-dependent Ca^{2+} entry via is involved in maintaining muscle mass.

Developing and differentiated muscle express different $Ca_V1.1$ splice variants

The idea that $Ca_V1.1$ could support substantial Ca^{2+} entry in myotubes was bolstered by the discovery of a high conductance embryonic/neonatal splice variant of $Ca_V1.1$ [158]. The newly described $Ca_V1.1e$ variant is missing exon 29, which encodes 19 residues within the short segment connecting helices S3 and S4 of the fourth conserved repeat (Fig. 1). qRT-PCR analysis of $Ca_V1.1$ transcript levels revealed that $Ca_V1.1e$ comprises nearly 80% of $Ca_V1.1$ expressed in wild-type myotubes. Although $Ca_V1.1e$ was able to engage EC coupling with similar efficiency as the original variant cloned from adult muscle ($Ca_V1.1a$) [1], L-type Ca^{2+} currents conducted by $Ca_V1.1e$ displayed very different properties (Fig. 5). Since $Ca_V1.1e$ activated at significantly more hyperpolarizing potentials and had an augmented P_o, the current was more than seven times larger than the $Ca_V1.1a$ variant. Block of Ca^{2+} entry with Cd^{2+} and La^{3+} also revealed a large component of Ca^{2+} transient that is a consequence of the current conducted by $Ca_V1.1e$. The findings of Tuluc et al. [158] not only explained why native L-type currents in myotubes are different in voltage-dependence and amplitude from *dysgenic* myotubes expressing adult rabbit $Ca_V1.1$ channels, but also demonstrated that native L-type currents in myotubes are capable of supporting robust Ca^{2+} entry [159].

Even before the absence of L-type current in *dysgenic* muscle was established [2], it was known that the diaphragms of *dysgenic* mice had altered innervation. In particular, *dysgenic* diaphragms display dispersed acetylcholine receptor (AChR) clusters rather than the central band of clusters present in diaphragm of normal mouse embryos [160-162]. Similarly, the diaphragms of β_1 null embryos display dispersed AChR clusters, and increased expression of both MuSK, an established promoter of AChR clustering, and AChRs [163]. Accompanying the changes in muscle, there is extensive sprouting of motor axons in both *dysgenic* [161] and β_1 null mice [163], whereas motor axons terminate at the central band of AChRs in wild-type mice. The muscle-specific re-introduction of β_{1a} (a transgenic technology that was unavailable in early characterizations of the *dysgenic* line) restores centrally located AChR clusters and, as a consequence, also eliminates the extensive nerve branching.

One common defect in these two effectively $Ca_V1.1$ null mouse models appears to be Ca^{2+} influx via $Ca_V1.1$. In particular, treatment of C_2C_{12} cells with the L-type channel antagonists isradipine and verapamil caused increased transcript levels of AChR and MuSK, whereas treatment with the L-type channel agonist (−)Bay K 8644 reduced the levels of both transcripts [163]. Interestingly, earlier work using a similar approach on cultured muscle cells had shown that the activity-dependent stabilization of AChRs at the endplate is dependent on L-type Ca^{2+} entry [164]. Taken together, these results provide support for the idea that the large Ca^{2+} currents produced by the embryonic/neonatal $Ca_V1.1e$ splice variant [158] may be important for normal embryonic and neonatal development [165].

Ca^{2+} influx via the $Ca_V1.1e$ variant may contribute to pathology of muscle in the multi-organ disorder, myotonic dystrophy type 1 (DM1). DM1 patients exhibit the re-emergence of the embryonic $Ca_V1.1e$ variant as a result of altered activity of the splice factors, MBNL1 and CUGBP1 [166]. Moreover, normal adult mouse *FDB* fibers expressing morpholinos designed to promote skipping of exon29 showed an increase in peak density, and hyperpolarizing shift in activation, of L-type Ca^{2+} current which are consistent with the behavior of $Ca_V1.1e$ expressed in *dysgenic* myotubes. The morpholino-treated *FDB* fibers also displayed enhanced voltage-evoked myoplasmic Ca^{2+} transients, which were shown by the addition of Cd^{2+} and La^{3+} to the external medium to contain a significant contribution from the L-type Ca^{2+} current. Thus increased entry of Ca^{2+} via $Ca_V1.1e$ represents a possible contributor to the dystrophic phenotype of DM1, an idea which is supported by the increased presence of central nuclei in *tibialis anterior* muscle of adult mice expressing $Ca_V1.1e$ [166].

Summary

The unique capability of $Ca_V1.1$ to serve as voltage sensor for EC coupling has long overshadowed its more traditional ability to conduct L-type Ca^{2+} current. However, the recent work indicating that Ca^{2+} does indeed enter muscle cells during depolarization through a dihydropyridine-sensitive pathway has given rise to a new curiosity about Ca 1.1 as a Ca^{2+} V channel. In this regard, the emergence of the high-conductance Ca 1.1e splice variant has demonstrated that Ca^{2+} V flux though the L-type channel can be rapid, substantial and physiologically relevant. Moreover, the reappearance of this embryonic variant in muscle of DM1 patients has shown that L-type Ca^{2+} entry certainly has pathological consequences.

Even though interest in $Ca_V1.1$ as channel has undergone a renaissance, the atypical role of EC coupling voltage sensor still commands more experimental attention, as it underlies an essential and conserved biological function. While there is consensus amongst investigators that the α_{1S} II-III loop and the β_{1a} subunit make indispensable contributions to EC coupling, the precise role(s) of these components of $Ca_V1.1$ remain enigmatic. In addition, the conformational changes that occur in the membrane-bound portions of the channel upon depolarization have not yet been characterized and how these molecular rearrangements are transduced to RyR1 to engage Ca^{2+} efflux from the SR is even more of a conundrum. Taken together, the investigation of these dual functions of the prototypical, yet most atypical, Ca_V channel will assuredly fascinate and frustrate muscle biologists for some time to come.

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Bannister and Beam Highlights

- This article reviews the dual functions of the skeletal muscle L-type Ca2+ channel.
- CaV1.1 is the voltage sensor for excitation-contraction coupling in skeletal muscle.
- CaV1.1 also conducts L-type Ca2+ current.
- Mutations in CaV1.1 have been linked to episodic muscle disorders.
- A CaV1.1 variant is involved in development and contributes to myotonic dystrophy 1.

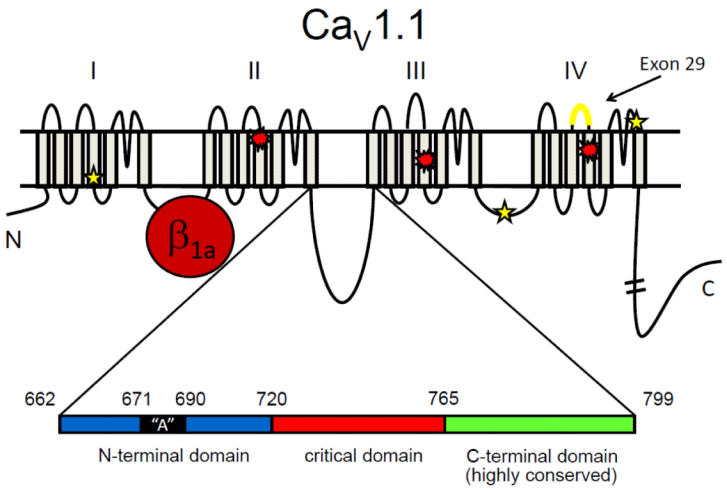

Fig. 1. Schematic representation of the Ca$_V$1.1

Cartoon illustrating the membrane topology of Ca$_V$1.1. Like Na$_V$ channels and the other nine members of the Ca$_V$ family, Ca$_V$1.1 is a single polypeptide composed of four relatively conserved repeats (I, II, II and IV) containing six α-helices apiece. The fourth α-helix of each has a regularly spaced sequence of basic residues that is believed to be critical for voltage-sensing. The segments linking the repeats, as well as the amino- and carboxyl-termini, are intracellular. The I-II-linker is the site of interaction with the predominantly intracellular β$_{1a}$ subunit (illustrated). The red box in the II-III loop represents the "critical domain" which is essential for engaging EC coupling (residues 720-765) [59]. The black box within the blue box represents the "A domain" (residues 681-690) [66,68]. The green box represents the highly conserved carboxyl-terminal domain [65]. The carboxyl terminus contains a proteolytic cleavage site at residue A1664 (hatch) [167], although this cleavage does not appear to affect the ability of Ca$_V$1.1 to couple to RyR1 [168]. The yellow segment indicates the position of an alternative splice (exon29) in the extracellular S3-S4 linker of Repeat IV [158]. The red explosions indicate known hypokalemic periodic paralysis (R528H, R900H and R1239H; please see text for references) mutations and the yellow stars signify residue substitutions that have been linked to malignant hyperthermia susceptibility (R174W, R1086H/C/S and T1354S; please see text for references).

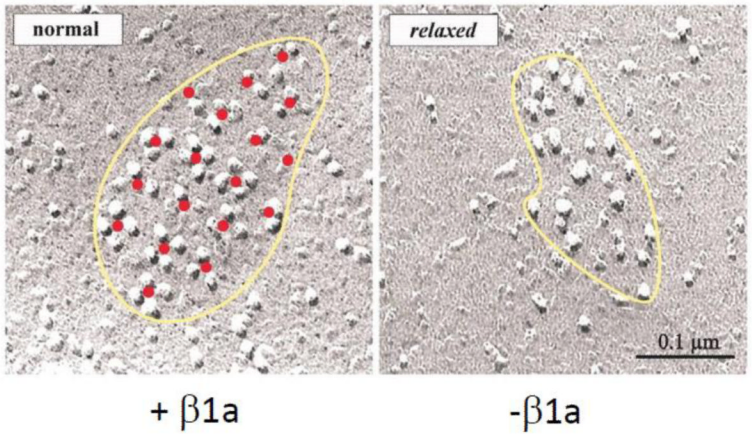

Fig. 2. Tetradic organization of $Ca_V1.1$ channels at plasma membrane junctions requires the $Ca_V\beta_{1a}$ subunit

Electron micrographs of freeze-fracture replicas of zebrafish membrane junctions are shown in both panels. In wild-type muscle (*left*), $Ca_V1.1$-containing channels exist in tetrads aligned with the four subunits of every other RyR1 homotetramer; each tetrad is highlighted by a red dot. In *relaxed*, or β_1 null, muscle, the $Ca_V1.1$ particles are sparse and tetrads are absent (*right*). Figure modified from Schredelseker et al. [30] with permission from the publisher; ©The Proceedings of the National Academy of Sciences of the USA, 2005.

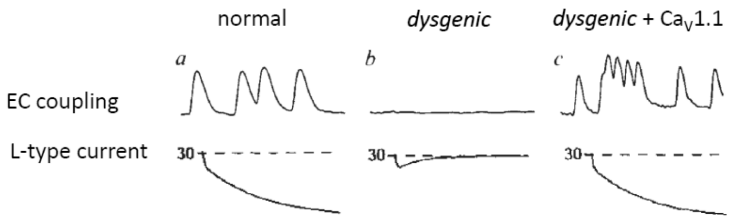

Fig. 3. Restoration of L-type current and EC coupling in *dysgenic* ($Ca_V1.1$ null) myotubes

EC coupling, as indicated by contractions elicited by focal electrical (*top* row), and Ca^{2+} currents recorded at +30 mV in the whole-cell configuration (*bottom* row) from normal myotubes (*left* panels), naïve *dysgenic* myotubes (*middle* panels) and $Ca_V1.1$-expressing *dysgenic* myotubes (*right* panels). Note the persistence of some T-type Ca^{2+} current in naïve *dysgenic* myotubes. Figure modified from Tanabe et al. [49] with permission from the publisher.

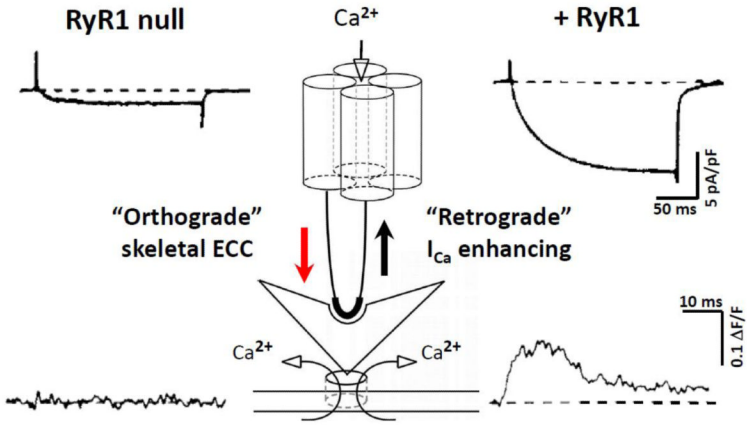

Fig. 4. Communication between $Ca_V1.1$ and RyR1 is bi-directional

The orthograde, or EC coupling, signal is communicated from $Ca_V1.1$ to RyR1. This signal is absent from *dyspedic* (RyR1 null) myotubes (*bottom left*). This signal is restored by reintroduction of the SR Ca^{2+} release channel (bottom right). Interestingly, *dyspedic* myotubes have meagre L-type Ca^{2+} current (*top left*), despite normal $Ca_V1.1$ expression. Reintroduction of RyR1 substantially increases L-type current density (*top right*), indicating that conformational coupling between $Ca_V1.1$ and RyR1 also produces a "retrograde" signal that serves to increase $Ca_V1.1$ relative P_o. Figure modified from Nakai et al. [88] and Grabner et al. [92] with permission from the publishers.

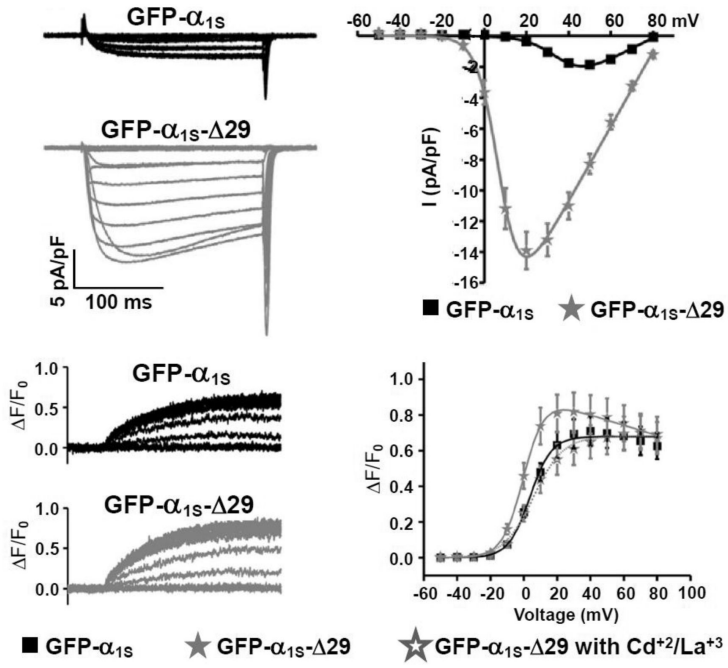

Fig. 5. A high-conductance $Ca_V1.1$ splice variant expressed in developing skeletal muscle

L-type currents recorded from *dysgenic* myotubes expressing either an adult α_{1S} isoform ($Ca_V1.1a$) [1] or an embryonic α_{1S} isoform lacking exon 29 [158] are shown in (A-*left*). A comparison of I-V relationships shows that $Ca_V1.1e$ has considerably larger current density and a hyperpolarizing shift in the voltage-dependence of activation relative to $Ca_V1.1a$ (A-*right*). Myoplasmic Ca^{2+} transients are augmented for $Ca_V1.1e$ (B-*left*), but the increase and hyperpolarizing shift in Fluo-4 signal represents the contribution of the L-type current because the ΔF/F-V relationship is nearly identical when Ca^{2+} entry via $Ca_V1.1$ is blocked by Cd^{2+} and La^{3+} (B-*right*). Figure modified from Tuluc et al. [158] with permission of the publishers; © Elsevier, 2009.