Modulation of L-type Ca2+ current but not activation of Ca2+ release by the gamma1 subunit of the dihydropyridine receptor of skeletal muscle

ABSTRACT

The L-type Ca2+ channel function, but not the SR Ca2+ release triggering function of the skeletal muscle dihydropyridine receptor, is modulated by the γ1 subunit.

INTRODUCTION

Background In skeletal muscle, the dihydropyridine receptor (DHPR) consists of α1S, α2-δ, β1a and γ1 subunits. This complex is responsible for the L-type Ca2+ current and serves as the voltage sensor for excitation-contraction (EC) coupling. In the latter process, the movement of electrical charges in the α1S subunit promotes a conformational change that opens the ryanodine receptor type-1 (RyR1) in the sarcoplasmic reticulum membrane (SR) leading to an increase in cytosolic Ca2+. The functional interactions between the DHPR subunits necessary for opening the Ca2+ channel are only partially known. Further, the interactions between DHPR subunits and RyR1 also are incompletely understood. The α1 subunit is a large protein that contains the basic functional elements of the L-type Ca2+ channel, including the Ca2+ selectivity, voltage-dependent gating, and sensitivity to dihydropyridines. The cytoplasmic loop between repeats II and III of the α1S subunit interacts closely with RyR1 and is an important determinant of skeletal type EC coupling. A region in the cytoplasmic loop between repeats I and II of the α1 subunit, referred to as the AID, binds tightly with a 30 amino acid region on the β1 subunit, referred to as the BID. β subunits are ~ 55 to 65 kDa proteins essential for channel assembly and/or membrane targeting, as well as for modulation of channel kinetics. The α2-δ subunit is a highly glycosylated ~ 175 kDa protein formed by two disulfide-linked peptides encoded by the same gene. Transmembrane topology and functional analyses suggest the α2-δ subunit is composed of a single transmembrane domain and a short cytoplasmic tail of only five residues. Given this topology, the α2-δ subunit is most likely to interact with the α1 and/or the γ1 subunits. The γ1 subunit is a ~ 32 kDa skeletal muscle-specific protein with four presumptive transmembrane domains. The transmembrane topology of the γ1 subunit and the critical binding domains are unknown at this time. EC coupling is initiated by voltage-dependent charge movements in the S4 segments of the DHPR α1S subunit, whose expression is dependent on the presence of the β1a subunit. The C-terminus of the β1a subunit has also been shown to be important in EC coupling, presumably by interaction with RyR1. The role of the α2-δ subunit on skeletal EC coupling is unknown, but in heterologous expression systems it has been demonstrated to increase the amount of charge movement. The role of the γ1 subunit in Ca2+ channel function is of particular interest given the discovery of a second γ subunit, γ2 or stargazin, which is expressed in neurons and is responsible for the stargazer mutation in mice. Subsequently, several other γ subunit genes have been identified and shown to be expressed in brain and peripheral tissues. In the present study we examined the role of the γ1 subunit in L-type Ca2+ current and EC coupling in skeletal myotubes. Absence of the γ1 subunit slows inactivation and produces a depolarizing shift in the Ca2+ current inactivation vs. voltage curve, in agreement with results from an independently produced γ1 knockout mouse. However, absence of γ1 does not affect the voltage dependence or the magnitude of charge movements and Ca2+ transients. Overall, the γ1 subunit appears to promote inhibition of the Ca2+ channel function of the skeletal DHPR. While this subunit is

clearly non-essential for activation of the L-type Ca2+ channel and for triggering skeletal-type EC coupling, γ1 appears to specifically modulate the Ca2+ channel function of the skeletal DHPR.

CONCLUSION

Conclusions The ability of the γ1 subunit to selectively modulate the pore function of the DHPR without modulation of charge movements or the voltage dependence of Ca2+ transients is unique, especially since other DHPR subunits participate in both functions. In all likelihood, the charge movement protocol failed to detect gating currents responsible for opening the Ca2+ channel, which are quite small and are only resolved for depolarizations >200 ms compared to the 25 ms used here (Fig. 4). A possible shift in the voltage-dependence of these charges recruited by long depolarizations would be consistent with the shift in Ca2+ current inactivation and remains to be resolved in γ1 null myotubes. However, the protocol accurately measures the immobilization-resistant charge movements that are known to be required for skeletal-type EC coupling. Therefore, the γ1 subunit is unlikely to play a critical role in the activation of SR Ca2+ release, in agreement with a recent report. However, shifts in voltage dependence below the limit of resolution (see Materials and Methods) and effects on charge movement and Ca2+ release inactivation cannot be completely ruled out. The γ1 knockout mice provide a unique resource to understand the function of this protein in myotubes in molecular detail.