

Modulation of L-type  $\text{Ca}^{2+}$  current but not activation of  $\text{Ca}^{2+}$  release by the  $\gamma 1$  subunit of the dihydropyridine receptor of skeletal muscle

## ABSTRACT

The L-type  $\text{Ca}^{2+}$  channel function, but not the SR  $\text{Ca}^{2+}$  release triggering function of the skeletal muscle dihydropyridine receptor, is modulated by the  $\gamma 1$  subunit.

## INTRODUCTION

Background In skeletal muscle, the dihydropyridine receptor (DHPR) consists of  $\alpha 1\text{S}$ ,  $\alpha 2\text{-}\delta$ ,  $\beta 1\text{a}$  and  $\gamma 1$  subunits. This complex is responsible for the L-type  $\text{Ca}^{2+}$  current and serves as the voltage sensor for excitation-contraction (EC) coupling. In the latter process, the movement of electrical charges in the  $\alpha 1\text{S}$  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  $\text{Ca}^{2+}$ . The functional interactions between the DHPR subunits necessary for opening the  $\text{Ca}^{2+}$  channel are only partially known. Further, the interactions between DHPR subunits and RyR1 also are incompletely understood. The  $\alpha 1$  subunit is a large protein that contains the basic functional elements of the L-type  $\text{Ca}^{2+}$  channel, including the  $\text{Ca}^{2+}$  selectivity, voltage-dependent gating, and sensitivity to dihydropyridines. The cytoplasmic loop between repeats II and III of the  $\alpha 1\text{S}$  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  $\alpha 1$  subunit, referred to as the AID, binds tightly with a 30 amino acid region on the  $\beta 1$  subunit, referred to as the BID.  $\beta$  subunits are ~ 55 to 65 kDa proteins essential for channel assembly and/or membrane targeting, as well as for modulation of channel kinetics. The  $\alpha 2\text{-}\delta$  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  $\alpha 2\text{-}\delta$  subunit is composed of a single transmembrane domain and a short cytoplasmic tail of only five residues. Given this topology, the  $\alpha 2\text{-}\delta$  subunit is most likely to interact with the  $\alpha 1$  and/or the  $\gamma 1$  subunits. The  $\gamma 1$  subunit is a ~ 32 kDa skeletal muscle-specific protein with four presumptive transmembrane domains. The transmembrane topology of the  $\gamma 1$  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  $\alpha 1\text{S}$  subunit, whose expression is dependent on the presence of the  $\beta 1\text{a}$  subunit. The C-terminus of the  $\beta 1\text{a}$  subunit has also been shown to be important in EC coupling, presumably by interaction with RyR1. The role of the  $\alpha 2\text{-}\delta$  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  $\gamma 1$  subunit in  $\text{Ca}^{2+}$  channel function is of particular interest given the discovery of a second  $\gamma$  subunit,  $\gamma 2$  or stargazin, which is expressed in neurons and is responsible for the stargazer mutation in mice. Subsequently, several other  $\gamma$  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  $\gamma 1$  subunit in L-type  $\text{Ca}^{2+}$  current and EC coupling in skeletal myotubes. Absence of the  $\gamma 1$  subunit slows inactivation and produces a depolarizing shift in the  $\text{Ca}^{2+}$  current inactivation vs. voltage curve, in agreement with results from an independently produced  $\gamma 1$  knockout mouse. However, absence of  $\gamma 1$  does not affect the voltage dependence or the magnitude of charge movements and  $\text{Ca}^{2+}$  transients. Overall, the  $\gamma 1$  subunit appears to promote inhibition of the  $\text{Ca}^{2+}$  channel function of the skeletal DHPR. While this subunit is

clearly non-essential for activation of the L-type  $\text{Ca}^{2+}$  channel and for triggering skeletal-type EC coupling,  $\gamma 1$  appears to specifically modulate the  $\text{Ca}^{2+}$  channel function of the skeletal DHPR.

## CONCLUSION

**Conclusions** The ability of the  $\gamma 1$  subunit to selectively modulate the pore function of the DHPR without modulation of charge movements or the voltage dependence of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  current inactivation and remains to be resolved in  $\gamma 1$  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  $\gamma 1$  subunit is unlikely to play a critical role in the activation of SR  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  release inactivation cannot be completely ruled out. The  $\gamma 1$  knockout mice provide a unique resource to understand the function of this protein in myotubes in molecular detail.