Excitation-Contraction Coupling Is Not Affected by Scrambled Sequence in Residues 681–690 of the Dihydropyridine Receptor II-III Loop*

Received for publication, July 14, 2000
Published, JBC Papers in Press, July 27, 2000, DOI 10.1074/jbc.C000464200

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A peptide corresponding to residues 681–690 of the II-III loop of the skeletal muscle dihydropyridine receptor α1 subunit (DHPR, α1S) has been reported to activate the skeletal muscle ryanodine receptor (RyR1) in vitro. Within this region of α1S, a cluster of basic residues, Arg681–Lys685, was previously reported to be indispensable for the activation of RyR1 in micromolar preparations and lipid bilayers. We have used an intact α1S subunit with scrambled sequence in this region of the II-III loop (α1S-scr) to test the importance of residues 681–690 and the basic motif for skeletal-type excitation-contraction (EC) coupling and retrograde signaling, as indicated by electrically evoked contractions and 0.5 mM Cd2+ and 0.1 mM La3+. The scrambled DHPR also rescued skeletal-type EC coupling, as indicated by electrically evoked contractions in the presence of 0.5 mM Cd2+ and 0.1 mM La3+. Furthermore, the release of intracellular Ca2+ was assayed by the indicator dye, Fluo-3, which had similar kinetics and voltage dependence for α1S and α1S-scr. These data suggest that residues 681–690 of the α1S II-III loop are not essential in muscle cells for normal functioning of the DHPR, including skeletal-type EC coupling and retrograde signaling.

* This work was supported by National Institutes of Health Grant NS24444 (to K. G. B.) with a minority supplement (for C. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: EC, excitation-contraction; DHPR, dihydropyridine receptor; RyR, ryanodine receptor; GFP, green fluorescent protein; PCR, polymerase chain reaction; F, farad(s).
Scrambled Sequence Does Not Affect DHPR Function

In this paper we have shown by expression in muscle cells that wild-type α1S and α1S-scr (α1S with scrambled sequence in residues 681−690 of the II-III loop) do not differ in density or voltage dependence of intracellular Ca2+ release. Thus, the function of α1S as a calcium channel and its ability to participate in EC coupling appear to be unaffected by either the specific sequence of residues 681−690 or the integrity of a cluster of basic residues in this region.

RESULTS

Macroscopic Ca2+ currents and intracellular Ca2+ transients were measured simultaneously (16) using borosilicate glass patch pipettes with resistances of 1.5−3.0 MΩ when filled with an internal solution containing (in mM) 1 MgCl2, 145 cesium glutamate, 10 HEPES, 2 CsCl, 0.1 EGTA, and 0.5 K2-Fluo-3 (Molecular Probes, Eugene, OR). The composition of the bath solution was 10 CaCl2, 145 tetraethylammonium chloride, 0.003 tetrodotoxin, and 10 HEPES (pH 7.4 with tetraethylammonium hydroxide). In some experiments, 0.5 mM CdCl2 and 0.1 mM LaCl3 were added to the extracellular solution. The voltage clamp command sequence was to step from a holding potential of −80 mV to −30 mV for 1 s, to −50 mV for 30 ms, to the test potential for 200 ms, and back to −80 mV. Test currents were digitally corrected for linear leakage and capacitative currents. Ca2+ currents were normalized by linear cell capacitance (pA/pF). All data are presented as mean ± S.E.

DISCUSSION

To test the importance of residues 681−690 for DHPR channel function, for retrograde signaling, and for EC coupling, we constructed a mammalian expression plasmid encoding the full-length pore-forming subunit of the skeletal DHPR (α1S) with a scrambled sequence in this region (α1S-scr, Fig. 1A). Note that the cluster of charged residues present in the wild-type sequence has been disrupted in α1S-scr. Whole-cell calcium currents recorded from dysgenic myotubes expressing α1S-scr closely resembled those recorded from myotubes expressing wild-type α1S (Fig. 1B) and, like the wild-type currents, were abolished by application of 0.5 mM Cd2+ and 0.1 mM La3+ (hatched bars). The cells were stimulated electrically (10 ms, 90 V), and the percentage observed to contract is indicated.

To assay the ability of α1S-scr to mediate skeletal-type EC coupling, dysgenic myotubes expressing either α1S or α1S-scr were tested both for contraction in response to extracellular stimulation and for depolarization-induced Ca2+ release. As illustrated in Fig. 2, wild-type α1S restored evoked contractions in 70% of fluorescent cells tested and α1S-scr restored contractions in 79% of cells tested. Myotubes expressing either α1S (67%) or α1S-scr (53%) retained the ability to contract even after the addition of 0.5 mM Cd2+ and 0.1 mM La3+ to the bathing medium to block Ca2+ entry.

Fig. 1. Scrambled sequence in the II-III loop does not alter calcium channel properties of the skeletal muscle dihydropyridine receptor. A, top, schematic illustration of the DHPR α1S subunit with the region of the II-III loop investigated indicated by a bold line. Bottom, sequence of residues 681−690 in the full-length α1S and α1S-scr constructs used in this study. B, representative whole-cell calcium currents recorded from dysgenic myotubes expressing α1S (left) or α1S-scr (right) in response to a voltage step to +40 mV. C, neither the average current density nor the voltage dependence of activation of α1S is altered by scrambled sequence in residues 681−690. Peak current densities for 14 cells expressing α1S (open circles) and 10 cells expressing α1S-scr (filled circles) are shown.

Fig. 2. Scrambled sequence in the II-III loop does not prevent the ability of DHPRs to restore EC coupling to dysgenic myotubes. Dysgenic myotubes expressing either α1S or α1S-scr were bathed in normal medium (gray bars) or in medium containing 0.5 mM Cd2+ and 0.1 mM La3+ (hatched bars). The cells were stimulated electrically (10 ms, 90 V), and the percentage observed to contract is indicated.
tal in origin (4, 5). The ability of these chimeras to produce skeletal coupling was independent of whether there was skeletal or cardiac sequence for residues 681–690. The interchangeability of cardiac and skeletal sequence in this region could simply be a consequence of sequence conservation or could mean that the sequence of these residues is unimportant for skeletal-type EC coupling. The present results strongly support the latter conclusion.

In vitro studies have shown that RyR1 is activated by a peptide composed of residues 681–690 (see Fig. 1A; Ref. 10) or by a slightly larger peptide (peptide A; residues 671–690 (7–9)). This activation is dependent on the integrity of a group of five basic residues within this region (10, 11). We have now shown that skeletal-type EC coupling still occurs in muscle cells expressing α1S bearing a scrambled sequence in the peptide A region, even though the same scrambled sequence abolished the ability of residues 681–690 to activate RyR1 (10). Thus it seems unlikely that in vitro activation of RyR1 by peptides implies in vivo activation of RyR1 by the corresponding region of the II-III loop. The activation by loop peptides may occur as a consequence of action at sites inaccessible to the intact II-III loop or may result from free solution conformations of the peptides that do not occur natively.

In conclusion, it is clear from our results that residues 681–690 are not required for EC coupling in vivo. However, our results do not exclude the possibility that residues 681–690 play some role in EC coupling, since moderate decreases in calcium release or efficacy of EC coupling would be difficult to detect with our methods.

Acknowledgments—We thank Katherine Parsons and Lindsay Grimes for expert technical assistance.

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