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Regulation of Muscle Cav1.1 Channels by Long-term Depolarization Involves Proteolysis of the α_{1s} Subunit

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Abstract. The effects of long-term depolarization on frog skeletal muscle Cav1.1 channels were assessed. Voltage-clamp and Western-blot experiments revealed that long-term depolarization brings about a drastic reduction in the amplitude of currents flowing through Cav1.1 channels and in the levels of the α_{1s} subunit, the main subunit of muscle L-type channels. The decline of both phenomena was prevented by the action of the protease inhibitors E64 (50 μM) and leupeptin (50 μM). In contrast, long-term depolarization had no effect on β_1, the auxiliary subunit of α_{1s}. The levels of mRNAs coding the α_{1s} and the β_1 subunits were measured by RNase protection assays. Neither the content of the α_{1s} nor the β_1 subunit mRNAs were affected by long-term depolarization, indicating that the synthesis of Cav1.1 channels remained unaffected. Taken together, our experiments suggest that the reduction in the amplitude of membrane currents and in the α_{1s} subunit levels is caused by increased degradation of this subunit by a Ca^{2+}-dependent protease.

Key words: Ca channels — Skeletal muscle — Channel turnover — Depolarization — Down-regulation — Calcium

Introduction

Skeletal muscle L-type Ca^{2+} channels play a fundamental role as the voltage sensors that link excitation with contraction (Lamb, 1992; Melzer, Hermann-Frank & Lüttgau, 1995). The channels are composed of several subunits. The α_{1s} subunit (or Cav1.1 channel, according to Ertel et al., 2000) is the main subunit, which contains the pore, the voltage sensor and the dihydropyridine binding sites, whereas α_2, β_1, γ and δ subunits are accessory subunits associated with α_{1s} (Catterall et al., 1993). In addition to their role as voltage sensors, Cav1.1 channels are permeant to Ca^{2+}, generating very slowly activated Ca^{2+} currents (Sanchez & Stefani, 1983). The role of the Ca^{2+} current in muscle is not well established. Recently, we have proposed that Ca^{2+} entry through these channels promotes down-regulation of the α_{1s} subunit. We have described that when muscle fibers are depolarized during a protracted period, a downregulation of the α_{1s} subunit takes place at the level of the cell membrane. Downregulation is prevented by the Ca^{2+} channel blocker nifedipine or in Ca^{2+}-free conditions (Escamilla et al., 2001). The present experiments further explore this phenomenon and show that this self-regulatory process is due to an increased degradation of the α_{1s} subunit and that no changes in channel synthesis are involved. Preliminary results have been published in abstract form (Carrillo et al., 2003).

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Materials and Methods

General Procedure

The experiments were performed in adult skeletal muscle fibers of the frog *Rana montezumae*. The frogs were sacrificed by decapitation. Long-term depolarization was achieved by incubating sartorius or semitendinosus muscle bundles in a solution containing 40 mM K^+ during 14 h at 4°C (*see* Solutions). After this period, the depolarizing solution was removed and the preparation was washed thoroughly with Ringer's, as detailed by Escamilla et al., (2001). Control experiments involved similar conditions, except that muscle fibers were maintained in the standard Ringer's solution. The protease inhibitors, E64 (Roche) or leupeptin (Roche) were used at a concentration of 50 μM. These inhibitors are permeant through cell membranes and their intracellular concentration, achieved after two hours of incubation, is sufficient to inhibit completely the intracellular activity of neutral proteases like

calpain (Atsma et al., 1995). These compounds were added to the incubation solutions as indicated. After this incubation period was over, the experiments were continued in the corresponding test solution at room temperature (20–25°C).

Ribonuclease Protection Assays

Total cellular RNA was isolated separately from each sartorius muscle bundle using the guanidinium isothiocyanate method (Chomczynski & Sacchi, 1987). Ribonuclease protection assays were carried out utilizing the RPA III Kit (Ambion), according to the manufacturer's instructions. The cDNAs used to synthesize the hybridization probes were the following: first, a frog 160 bp cDNA corresponding to intracellular ll-lll loop of the skeletal DHPR α_1-subunit, specific for the skeletal muscle isoform. This was produced by PCR using the following primers: Fα 5′ GCG AAT CCA GAC AAA ACA GAG GAA 3′ and Rα5′ TGG GAA ATC TGC AGA GGG ATA AGG 3′; second, a 138 bp corresponding to the beta subunit of skeletal muscle produced by PCR with the primers: Fβ 5′TGA ATT CTT TAA GAA GAC AGA GCA 3′ and Rβ 5′ATG GTC GAG GAA GTC AAA CAA 3′ and finally, a 584 bp fragment corresponding to beta actin produced by PCR amplification with the primers: Fa 5′ CCA AGG CCA ACC GCG AGA AGA TGA C 3′ and Ra 5′ AGG GTA CAT GGT GGT GCC GCC AGA C 3′. This fragment was used as an internal marker for the standardization of the amount of RNA, and this product, as well as all others, was cloned in a pBluescript KS+ vector (Stratagene). The clones were sequenced (ABI PRISM Model 310 Perkin Elmer). The RNA antisense probes were obtained with the MAXIscript In Vitro transcription kit (Ambion) utilizing linearized plasmids in the presence of [^{35}S]rUTP (Amersham). 10 μg of total RNA from muscles incubated during 14 h in Ringer's, in high K^+ or in high K^+ plus nifedipine were hybridized with different RNA probes. The analysis of protected fragments was done in 6% polyacrylamide/8 M urea gels. Gels were fixed, dried and were used to expose X ray film. Autoradiograms of RNAse protection assays were subjected to densitometric analysis using the KodaKID Image Analysis Software. The intensity of hybridization with the skeletal muscle DHPR was corrected for variations in gel loading by normalizing to the intensity of hybridization of the same RNAs to the RNA (RNase protection assay) probe for beta actin.

Western Blots

Crude skeletal muscle membranes were prepared as described elsewhere (Escamilla et al., 2001). The α_{1s} and β_{1a} subunits and the structural protein caveolin were identified by Western blotting. Western blots were performed by applying 6–15 μg of each crude membrane preparation to a 7.5% (for α_{1s}- and β_{1a}-subunit immunoblots) or 13% (for caveolin immunoblots) sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel. The gel proteins were then transferred to a nitrocellulose membrane and probed with primary antibodies followed by a secondary antibody and, finally, detected by enhanced chemiluminescence using an ECL detection kit (Amersham). The blots were blocked with 5% nonfat dried milk in phosphate buffered saline (PBS). The primary antibodies used in this study were: an anti-pan α_1 [anti-CP-(1382–1400)], from Alomone Labs, Jerusalem, Israel, used at a 1:300 dilution, that recognizes a highly conserved region of the α_{1s} subunit (residues 1382–1400 located intracellularly in the C-terminal part) and an antibody that recognizes the skeletal muscle DHPR β_1 subunit on Western blots (Upstate Biotechnology, USA), used at a 1:1000 dilution. After washing, the membranes were incubated with an anti-rabbit (1:2500) or an anti-mouse (1:3000) horseradish peroxidase conjugate (Amersham Life Sciences) as secondary antibodies. The densities of the bands corresponding to α_{1s} or β_1 subunits were measured with the KodaKID Image Analysis Software.

As internal control, the levels of caveolin, a structural protein, were assessed in immunoblots. The gel proteins were probed with Caveolin 1/3 Polyclonal Antiserum (Cayman) as the primary antibody, used at 1:1600 dilution. This antibody recognizes caveolin 1 (22–25 kDa) and caveolin 3 (20–22 kDa). Both isoforms are expressed in skeletal muscle (Oka et al., 1997). An anti-rabbit (1:2500) horseradish peroxidase conjugate (Amersham Life Sciences) was used as secondary antibody.

Solutions

The standard Ringer's contained (mM): NaCl, 117; KCl, 2.5; $CaCl_2$, 1.8. The depolarizing solution used contained (mM): Na^+, 42.3; K^+, 40; Ca^{2+}, 7.6; SO_4^{2-}, 45 and Cl^-, 7.6. This K^+ concentration was chosen because it produces near-maximal effects down-regulating Ca channels of neurons (Liu et al., 1994) and muscle (Escamilla et al., 2001). Nifedipine (Sigma-Aldrich) solutions were prepared from a concentrated DMSO stock solution and was used at a concentration of 20 μM. The nifedipine experiments were conducted in the dark. The extracellular solution employed for measurements of Ca-channel currents contained (mM): TEA, 110; Ba^{2+}, 10 and methanesulfonate ($CH_3SO_3^-$) as anion. The intracellular solution contained (mM): TEA, 154; EGTA, 77 and ATP (Na_2), 2.5. Ba^{2+} was replaced by Mg^{2+} (10 mM) in charge-movement experiments. Extracellular and intracellular solutions were buffered with 3-N-morpholino propanesulfonic acid (MOPS) at pH = 7.2 and 7.1, respectively.

Voltage-Clamp Experiments

We followed the procedure described in detail by Escamilla et al. (2001). In brief, small bundles of intact muscle fibers from semitendinosus muscle were exposed to the depolarizing test solution for 14 h. After the depolarizing period was over, the bundle was washed thoroughly in Ringer solution and one fiber was selected and mounted in the voltage-clamp chamber, at which time the fiber was cut. Membrane currents were measured in cut fibers using the triple-Vaseline gap voltage clamp technique (Hille & Campbell, 1976).

The protocol to measure L-type currents and non linear capacitive currents is described elsewhere (Escamilla et al., 2001). To calculate charge movement, "on" currents were integrated and the data points were fitted to a two-state Boltzmann distribution:

$$Q = Q_{max}/[1 + \exp((-V_m + V)/k)] \quad (1)$$

where Q_{max} is the maximum charge per unit linear capacitance, V is the midpoint and k is a measure of the steepness.

Data are expressed as mean values ± SEM. The statistical significance of differences in mean values was assessed by the unpaired t-test. A value of $P < 0.05$ level was considered to be significant.

Results

Actions of Long-term Depolarization on mRNA Levels

We found no changes in the expression levels of the mRNA of the α_1 subunit of Cav1.1 channels. This is illustrated in Fig. 1, which shows that the transcript, specific of the skeletal Cav1.1 channel, is unaffected

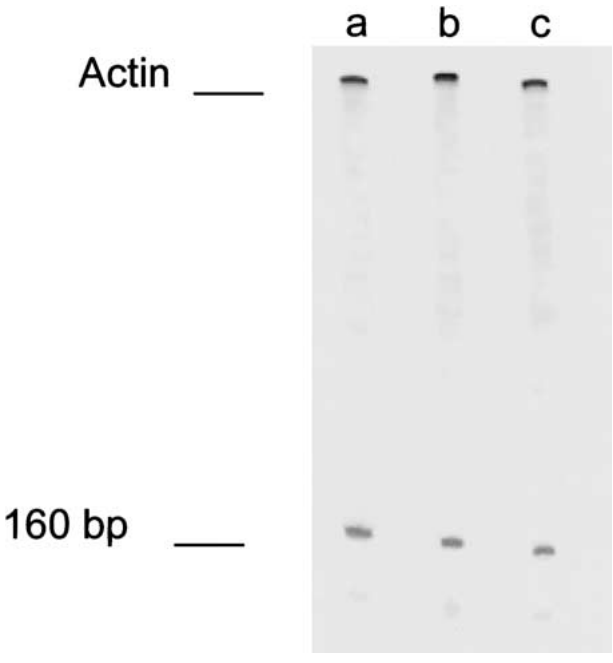

Fig. 1. The effects of long-term depolarization on the expression of muscle α_{1s} subunit mRNA. Illustrated are representative RNase protection assays demonstrating the presence of the expected 160 bp protected fragment. No decrease in the expression was seen 14 h after depolarization with high K^+ (*b*) when compared with the expression after 14 h in the control solution (*a*) or in high K^+ plus nifedipine (*c*).

by preincubation in the high-K^+ depolarizing solution. Likewise, blocking the entry of Ca^{2+} ions through L-type channels with the specific blocker nifedipine had no influence on mRNA expression. As expected, the levels of mRNA of β-actin remained unchanged under these experimental conditions. The lack of effect of long-term depolarization on the messenger levels of the α_{1s} subunit was confirmed quantitatively when the densities of the band corresponding to the 160 bp fragment were compared with those of the β-actin fragment. Thus, in five separate experiments, these ratios averaged 0.71 ± 0.05 under control conditions, 0.68 ± 0.05 in preincubated fibers in high K^+ and 0.68 ± 0.05 when nifedipine was added to the depolarizing solution. These values were not statistically different ($P > 0.05$).

We next explored whether long-term depolarization changes the levels of mRNA of the β_1 subunit, one of the auxiliary subunits of Cav1.1 channels. This determination was especially important because the β_1 subunit plays a key role in the trafficking and incorporation of Cav1.1 channels to the muscle plasma membrane (Gregg et al., 1996). Therefore, a decrease in the level of this subunit or in its corresponding mRNA might in turn decrease the level of the α_{1s} subunit produced by long-term depolarization described below. RNase protection assay experiments revealed that no changes were produced at the level of the β_1 messenger. Thus, in three experiments, the ratio between the density of the band corresponding to the messenger of the β_1 subunit and that of the messenger of the β-actin averaged 0.97 ± 0.09 in control experiments, 0.94 ± 0.06 under depolarizing conditions and 0.95 ± 0.07 when nifedipine was present in the high-K^+ solution. These values were not statistically different ($P > 0.05$).

Actions of Long-Term Depolarization on Cav1.1 Channels at the Protein Level

Long-term depolarization decreases the levels of the α_{1s} subunit. Figure 2*A* shows a 170 kDa band detected by the anti-α_{1s} antibody in the control experiment (lane *a*), the size of the majority of the skeletal α_{1s} subunits in adult skeletal muscle (De Jongh, Merrick & Catterall, 1989). The expression of the skeletal muscle α_{1s} subunits greatly decreased in fibers that had been previously incubated for 14 h in high K^+ (lane *b*). The relative density values (high K^+/control) significantly decreased to 0.33 ± 0.03 ($n = 8$) ($P < 0.05$), confirming previous results (Escamilla et al., 2001). To determine whether the drip in α_{1s} subunit levels is due to increased proteolysis, muscle fibers were incubated in the depolarization solution as before, except that the protease inhibitor E64 was added. We found that E64 greatly prevented the decline in the expression of the α_{1s} subunit brought about by long-term depolarization (lane *c*). The corresponding ratio averaged 0.88 ± 0.02 ($n = 8$). Similar results were observed with the protease inhibitor leupeptin: 0.83 ± 0.02 ($n = 8$).

In contrast to the decrease of the α_{1s} subunit, we found no changes in the levels of the auxiliary β_1 subunit, as detected by Western blot. Figure 2*B* shows representative results. A distinct band with a molecular weight slightly below 50 kDa was identified, whose intensity was not changed by long term depolarization. The relative density values: high K^+/control and high K^+ plus E64/control, averaged 1.0 ± 0.01 ($n = 4$) and 0.97 ± 0.01 ($n = 4$), respectively, confirming that preincubation in a high-K^+ solution has no effect on the β_1 subunit at the protein level.

Long-term depolarization does not appear to lead to widespread proteolysis in muscle. Figure 2*C* shows representative immunoblots of the structural protein caveolin. The same crude membrane extracts used in the experiments of Fig. 2*A* and *B* were probed. Two distinct protein bands were detected, whose molecular weights were 25 kDa and 22 kDa, corresponding to caveolin 1 and 3, respectively. The density of both bands remained unaffected by preincubation in high K^+. The relative densities of the bands (K^+/control) were: 0.97 ± 0.01 ($n = 5$) and 0.97 ± 0.01 ($n = 5$) for caveolin 1 and 3, respectively. A similar comparison of the same bands in

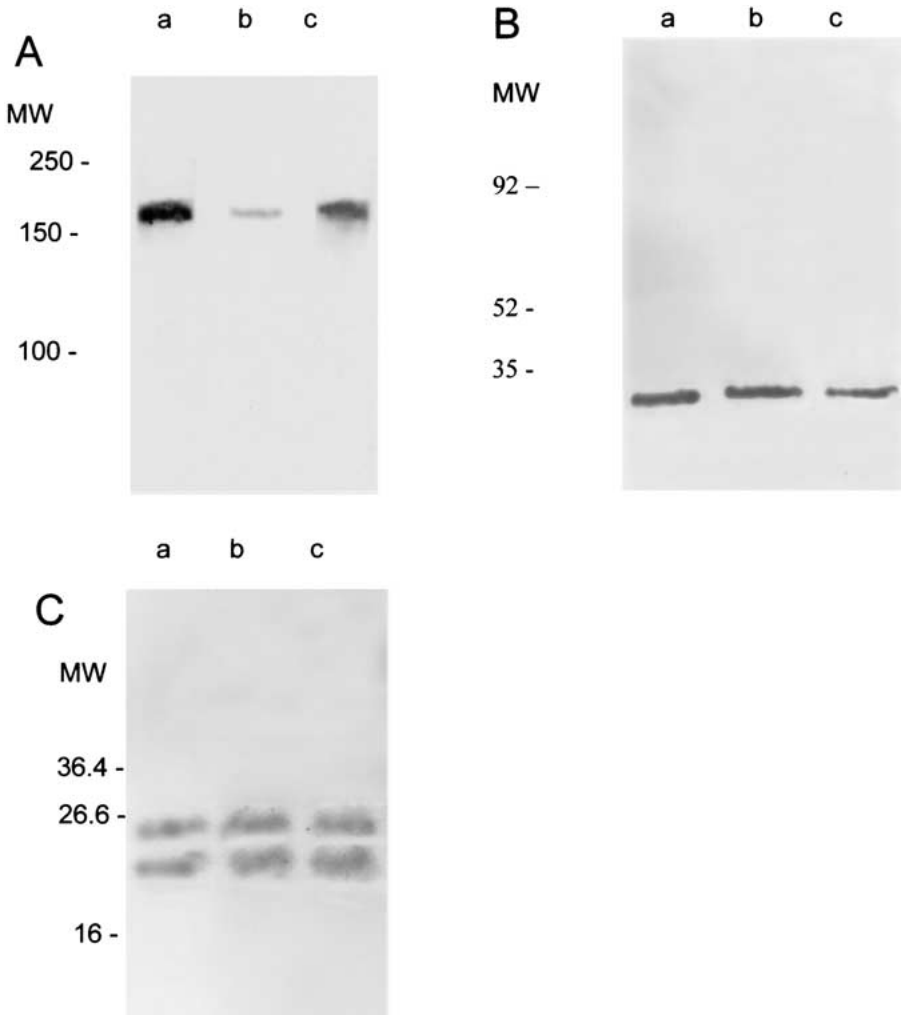

Fig. 2. The effects of long-term depolarization on α_{1s} and β_1 subunits at the protein level. (*A*) The blots show that an anti-pan α_1 antibody recognizes a single band at approximately 170 kDa. The band was thick under control conditions (*a*) and faint in previously depolarized cells (*b*), an effect that was prevented in part by the presence of E64 in the depolarizing solution (*c*). (*B*) The blots show that the anti-β_1 antibody recognizes a single band under control conditions (*a*) whose intensity did not change by long-term depolarization (*b*) or when E64 was present in the high-K^+ solution (*c*). (*C*) The blots show that the anti caveolin antibody recognizes two bands under control conditions (*a*) whose intensity did not change by high-K^+ predepolarization (*b*) or by high-K^+ predepolarization plus E64 (*c*).

the presence of K^+ plus E64 or of K^+ plus leupeptin gave similar results: 0.98 ± 0.01 ($n = 5$) and 0.99 ± 0.01 ($n = 5$) for E64 and 0.96 ± 0.01 ($n = 5$) and 0.98 ± 0.01 ($n = 5$) for leupeptin.

Protease Inhibitors Prevent the Decline of Ca^{2+} Channel Current Amplitude by Long-term Depolarization

Consistent with the action of protease inhibitors that prevented the decline in the level of the α_{1s} subunit described above, we found that they also opposed the decrease of L-type currents produced by long-term depolarization. Figure 3*A* shows representative control membrane currents generated during depolarizing pulses to +5, +15 and +20 mV. Panel *B* shows current records from a separate experiment to the same potentials from a fiber that was previously incubated in the depolarizing solution. Currents were smaller and activated following a slower time course, as described previously (Escamilla et al., 2001). Panel *C* corresponds to a fiber that was preincubated in a depolarizing solution as in *B*, but that also contained the protease inhibitor E64. Clearly, preincubation with E64 prevented the reduction that long-term depolarization produces in the amplitude of L-type currents as well as the changes in their time course. Figure 3*D* shows the mean current-voltage relation from several experiments similar to those shown in Fig. 3*A–C*. To compare data from different experiments, peak current values were normalized to unit capacitance. Under control conditions (○), currents peaked at +5 mV, reaching a value of -45.1 ± 2.3 $\mu A \cdot \mu F^{-1}$ ($n = 9$) and had an extrapolated reversal potential more positive than +60 mV. Currents recorded from predepolarized fibers (●) were significantly smaller at all potentials, reaching a peak value of -17.6 ± 0.5 $\mu A \cdot \mu F^{-1}$ ($n = 12$) at +10 mV. When fibers were pre-incubated in the depolarizing solution in the presence of the protease inhibitor E64, the reduction in current amplitude was largely prevented, as shown in Fig. 3 (▼). The current-voltage relation reached a value of -41.8 ± 2.8 $\mu A \cdot \mu F^{-1}$ ($n = 12$). We verified that E64, by itself, did not alter the magnitude of Ca^{2+}-channel currents either in acute experiments or when fibers were preincubated

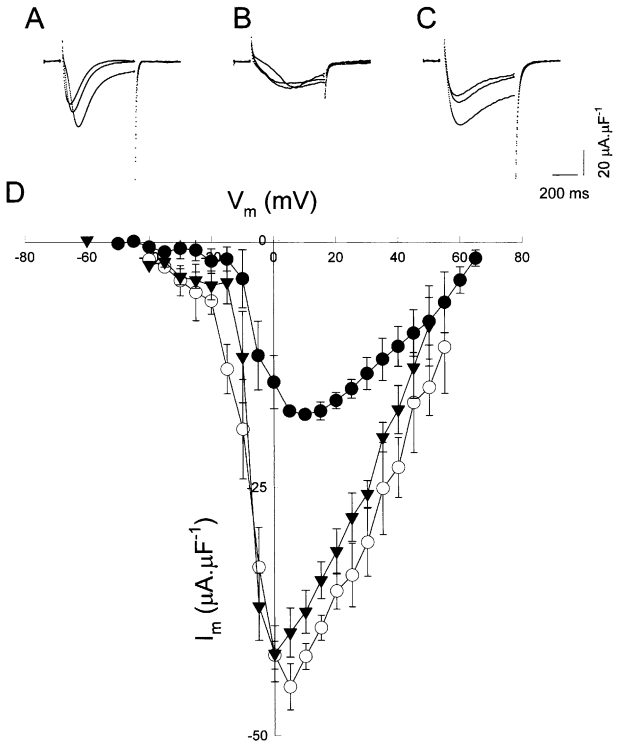

Fig. 3. The effects of long-term depolarization on muscle L-type currents. (*A–C*) Three separate experiments illustrating L-type channel currents recorded during pulses to +5, +15 and +20 mV. In *A*, the fiber was preincubated in Ringer's for 14 h. In *B*, the fiber was preincubated in a depolarizing solution containing 40 mM K^+. In *C*, the fiber was preincubated in 40 mM K^+ plus E64. $E_h = -90$ mV. (*D*) The current-voltage relation of L-type currents. Mean values (±SE) of peak currents recorded after preincubation of muscle fibers for 14 h under control conditions, as in *A* (○); data from fibers preincubated as in *B* (●) and results from experiments as in *C* (▼).

in Ringer's to which this protease inhibitor was added. Thus, in acute experiments, peak currents averaged -44.2 ± 2.7 ($n = 3$) $\mu A \cdot \mu F^{-1}$.

Although the fall in the level of the α_{1s} subunit and in the amplitude of the Ca^{2+}-channel currents was greatly prevented by E64, the protein inhibitor was not equally effective: peak current density reached 93% of its control value when E64 was added to the depolarizing solution. The corresponding figure for the α_{1s} subunit was 88%. The reason for this small discrepancy is presently unknown.

Consistent with the actions of protease inhibitors preventing the reduction of L-type currents that long-term depolarization brings about, we found that E64 is equally effective in preventing the decline in charge movement. Table 1 shows the average values of the Boltzmann parameters from control experiments, from long-term depolarized fibers and from fibers that were depolarized in the presence of E64. The maximum value of charge was reduced by 60% by long-term depolarization, an effect that was fully prevented by E64. No significant changes in the mid-

Table 1. Actions of long-term depolarization on charge movement

	Q_{max} (nC μF^{-1})	V (mV)	k (mV)	N
Control	34.2 ± 4.1	−32.7 ± 2.4	8.4 ± 0.8	5
K^+	13.6 ± 3.5	−33.1 ± 1.5	7.5 ± 0.7	7
E64 plus K^+	38.1 ± 3.9	−30.3 ± 2.3	9.3 ± 0.9	9

value of activation or in the slope were observed. The possibility that the decrease in the amplitude of L-type currents is due to detachment of the transverse tubular system membranes (where Cav1.1 channels are located) from the surface membrane was ruled out by measuring C_m ($\mu F \cdot cm^{-2}$), the specific membrane capacitance of muscle fibers under different experimental conditions. Under control conditions, $C_m = 7.6 \pm 1.8$ ($n = 4$); after long-term depolarization, $C_m = 7.4 \pm 0.4$ ($n = 4$); in the presence of E64 in the high-K^+ solution, $C_m = 7.8 \pm 1.2$ ($n = 7$). No statistical difference was found in these determinations.

Discussion

Downregulation of Ca^{2+} Channels by Prolonged Depolarization

Ca^{2+} entry through L-type Ca^{2+} channels of excitable cells plays a fundamental role in physiological phenomena like contraction in heart and smooth muscle and as a link to transduce depolarization into non-electrical activities (for reviews see Hille, 2001; Bers, 2002; Ganitkevich, Hasse & Pfitzer, 2002). Ca^{2+} influx by prolonged polarization also leads to a regulatory process that brings about a reduction in the amplitude of L-type currents of neurons, muscle fibers and other excitable cells (De Lorme, Rabe & McGee, 1988; Franklin, Fickbohm & Willard, 1992; Liu et al., 1994; Escamilla et al., 2001). Loss of functional Ca^{2+} channels by depolarization in muscle and other cells is accompanied by a decrease in the levels of the α_1 subunit located at the cell membrane (Liu et al., 1994; De Lorme et al., 1998; Escamilla et al., 2001). This fact suggests that the underlying mechanism involves either an increase in the degradation of the channels or a decrease in their synthesis. Regarding the last possibility, previous work has shown that an increase in cytosolic Ca^{2+} downregulates the number of Na^+ channels and the associated α subunit mRNA levels (Offord & Catterall, 1989; Shiraishi et al., 2001). The possibility that the synthesis of Ca^{2+} channels decreases by a prolonged depolarization has not been previously explored in muscle, and experiments in other systems have given conflicting results. Thus, Liu, Rutledge & Triggle (1995) found no changes in the content of mRNA encoding the α_1 subunit of GH_4C_1 cells. In contrast,

Feron and Godfraind (1995) reported a drop in mRNA expression by prolonged depolarization in PC12 cells. Differences in cell types, experimental conditions or techniques might explain these discrepancies. Our present results clearly show that Cav1.1 channels of adult skeletal muscle are downregulated without changes in the levels of mRNAs coding the α_{1s} or the β_1 channel subunits.

Prolonged Depolarization Produces Proteolysis of Muscle Cav1.1 Channels

The action of protease inhibitors on channel downregulation that we report here, points to an increased degradation of the channels as the underlying mechanism that decreases the levels of the α_{1s} subunit after a prolonged depolarization.

Proteolysis of the channel produced a single band of decreased density in our immunoblots, which corresponds to the intact α_{1s} subunit. This indicates that no cleaved products of proteolysis of the α_{1s} subunit, containing the epitope, remained attached to the fiber membrane. Since the epitope recognized by the antibody is located in the C-terminal region, our present results would be explained if cleavage at this point, or further upstream in the C-terminal domain, is taking place. A cleavage at this level would be expected to detach the distant C-terminal domain from the rest of the channel and from the membrane. This does not rule out the possibility that other regions of the α_{1s} subunit are also cleaved as well. However, at least one region of the α_{1s} subunit remains unaffected, the alpha interaction domain (AID), a highly conserved site in the α_{1s} subunit to which the β_1 subunit binds (Pragnell et al., 1994). Cleavage at this point would be expected to dissociate these two subunit from each other, leading to a decrease in the membrane levels of the β_1 subunit, a decrease that was not observed. Also, the fact that long-term depolarization does not lead to downregulation of the β_1 subunit suggests that no extensive proteolysis is taking place. It is therefore plausible that proteolysis involves specific regions of the α_{1s} subunit only. In this regard, it is interesting to note that some proteases cleave proteins near peptide motifs that serve as proteolytic signals. These include PEST regions and KFERQ motifs (Rechsteiner & Rogers, 1996).

Although our experiments support the involvement of a protease in the downregulation of Cav1.1 channels by depolarization, an exact description of the proteolytic process of the α_{1s} subunit remains to be unveiled. Furthermore, we have no direct evidence on the nature of the protease involved. However, previous work indicates that downregulation strictly requires the entry of Ca^{2+} through muscle α_{1s} channels (Escamilla et al., 2001). Considering this fact and the present results, it is likely that downregulation in muscle depends on the activation of a Ca^{2+}-dependent protease located very near the channel mouth. This conclusion is based on the fact that myoplasmic Ca^{2+} is well buffered by the sarcoplasmic reticulum and by Ca^{2+}-binding proteins that would prevent the free diffusion of this cation far from the plasma membrane. The localization of Ca^{2+}-activated proteases at the level of the *Z* line (Dayton & Schollmeyer, 1981; Kumamoto et al., 1992), in the vicinity of the transverse tubular system where Cav1.1 channels are located (Fosset et al., 1983), further supports the suggestion that a protease activated by Ca^{2+} ions flowing through these channels plays a key role in the turnover of the protein. A possible candidate is calpain, a member of the Ca^{2+}-dependent proteases (Goll et al., 2003; for reviews on calpain *see* Croall & De Martino, 1991; Sorimachi & Suzuki, 2001), which is inhibited by E64 and leupeptin (Atsma et al., 1995). Among the different calpains, skeletal muscle expresses a tissue-specific calpain, p94 or calpain 3a, at a level ~10 times higher than the levels of the mRNAs for other calpains (Kinbara et al., 1998; Goll et al., 2003).

Run-down of Ca^{2+}-Channel Activity

The presence of Ca^{2+}-dependent proteases in cells requires a tight regulation of their proteolytic activity. Otherwise, proteolysis of channels and other targets would severely compromise cell function. It is therefore not surprising that when the intracellular environment is disturbed, Ca^{2+} channel activity runs down. Chad & Eckert (1986) described that the irreversible development of run-down of Ca^{2+} currents of snail neurons is greatly prevented by the protease inhibitor leupeptin. The run-down of Ca^{2+} channels of heart muscle is partially recovered by addition of calpastatin, an endogenous inhibitor of calpain, leading to the conclusion that proteolytic degradation of Ca^{2+} channels is the cause of the run-down phenomenon (Belles et al., 1988; Romanin et al., 1991; Hao et al., 2000). Cav1.1 channels of skeletal muscle also run down in cut fibers by a process that cannot be entirely explained by dephosphorylation (Arreola et al., 1987). It is therefore plausible that muscle proteases are also involved in this process.

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