Reduced Ca^{2+} Current, Charge Movement, and Absence of Ca^{2+} Transients in Skeletal Muscle Deficient in Dihydropyridine Receptor β_1 Subunit

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ABSTRACT The Ca^{2+} currents, charge movements, and intracellular Ca^{2+} transients in mouse skeletal muscle cells homozygous for a null mutation in the *cchb1* gene encoding the β_1 subunit of the dihydropyridine receptor have been characterized. $I_{\beta null}$, the L-type Ca^{2+} current of mutant cells, had a ~13-fold lower density than the L-type current of normal cells (0.41 ± 0.042 pA/pF at +20 mV, compared with 5.2 ± 0.38 pA/pF in normal cells). $I_{\beta null}$ was sensitive to dihydropyridines and had faster kinetics of activation and slower kinetics of inactivation than the L-type current of normal cells. Charge movement was reduced ~2.8-fold, with Q_{max} = 6.9 ± 0.61 and Q_{max} = 2.5 ± 0.2 nC/μF in normal and mutant cells, respectively. Approximately 40% of Q_{max} was nifedipine sensitive in both groups. In contrast to normal cells, Ca^{2+} transients could not be detected in mutant cells at any test potential; however, caffeine induced a robust Ca^{2+} transient. In homogenates of mutant muscle, the maximum density of [^{3}H]PN200-110 binding sites (B_{max}) was reduced ~3.9-fold. The results suggest that the excitation-contraction uncoupling of β_1-null skeletal muscle involves a failure of the transduction mechanism that is due to either a reduced amount of α_{1S} subunits in the membrane or the specific absence of β_1 from the voltage-sensor complex.

INTRODUCTION

The dihydropyridine (DHP) receptor complex of skeletal muscle comprises four subunits: α_{1S}, β_1, α_2/δ, and γ. This complex serves as a voltage sensor during excitation–contraction (E-C) coupling and is responsible for the L-type Ca^{2+} current. The α_1 subunit of the DHP receptor contains the basic functional elements of the L-type Ca^{2+} channel, which include a selectivity filter, a voltage sensor, and binding sites for DHPs. The β subunit is located intracellularly, and, in skeletal muscle, β_1 consists of 524 amino acids (Ruth et al., 1989). Transcripts from three additional genes encoding β subunits have been reported (Hullin et al., 1992; Castellano et al., 1993). Alternate splicing of the primary β_1 gene (*cchb1*) transcript produces at least three isoforms. One (β_{1a}) is expressed exclusively in skeletal muscle, whereas the other two (β_{1b}, β_{1c}) are expressed in brain, heart, and spleen (Powers et al., 1992).

The L-type Ca^{2+} current generated by interactions between specific subunits has been studied with a variety of heterologous expression systems (for a review, see Hofmann et al., 1994). Coexpression of the β_1 subunit with either cardiac (α_{1C}) or skeletal (α_{1S}) α_1 subunits produces an increase in L-type Ca^{2+} current density (Singer et al., 1991; Wei et al., 1991; Itagaki et al., 1992; Lory et al., 1992, 1993; Nishimura et al., 1993; Perez-Garcia et al., 1995; Josephson and Varadi, 1996; Kamp et al., 1996), an increase in the inactivation rate (Singer et al., 1991; Varadi et al., 1991; Lory et al., 1993; Stea et al., 1993; De Waard et al., 1994; Perez-Garcia et al., 1995), an increase in maximum DHP binding site density (Lacerda et al., 1991; Varadi et al., 1991; Wei et al., 1991; Lory et al., 1992; Nishimura et al., 1993; Perez-Garcia et al., 1995), and a decrease in Ca^{2+} current sensitivity to Bay K 8644 (Singer et al., 1991; Varadi et al., 1991; Itagaki et al., 1992; Lory et al., 1992). The effect on the activation rate and the DHP binding affinity varies, depending on the expression system. Some investigators found an increase in the activation rate (Lacerda et al., 1991; Singer et al., 1991; Varadi et al., 1991; Wei et al., 1991; Lory et al., 1992); however, others found no effect (Itagaki et al., 1992; Lory et al., 1993) or even a decrease in the activation rate (Perez-Garcia et al., 1995). Further, some investigators found an increase in DHP binding site affinity (Mitterdorfer et al., 1994), whereas the others found no change (Lacerda et al., 1991; Varadi et al., 1991; Perez-Garcia et al., 1995). Whether β_1 modulates α_{1S} function in muscle cells in a manner that is analogous to the findings in heterologous systems is unknown.

In recent years, the role of α_1 subunits in E-C coupling has been established by the use of dysgenic mice characterized by the absence of the α_{1S} and the absence of E-C coupling (Tanabe et al., 1988, 1990a,b; Adams et al., 1990). Expression studies in dysgenic skeletal muscle cells demonstrated the importance of the cytoplasmic loop between repeats II and III of α_{1S} for skeletal-type E-C coupling (Tanabe et al., 1990a). To determine the specific roles of β_1 in skeletal-type E-C coupling and the modulation in this tissue of the L-type Ca^{2+} current by β_1, Gregg et al. (1995,

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1996) used gene targeting to inactivate the murine β_1 gene (*cchb1*). Mice heterozygous (*cchb1*$^{+/-}$) for the mutation develop normally. Mice homozygous (*cchb1*$^{-/-}$) for the mutation die from asphyxia at birth because of the lack of E-C coupling in skeletal muscles. In the present study we characterized Ca^{2+} currents, intramembrane charge movements, and intracellular Ca^{2+} transients in β_1-null myotubes. Part of this research appeared previously in abstract form (Strube et al., 1995, 1996; Beurg et al., 1996).

MATERIAL AND METHODS

Single-cell preparation

All experiments were performed on freshly isolated intercostal myotubes from 18-day-old mouse fetuses. Homozygotes (*cchb1*$^{-/-}$) for the mutation are hereafter called mutant or β_1-null (Gregg et al., 1996). Fetuses with a normal phenotype, hereafter called normal, were either heterozygotes (*cchb1*$^{+/-}$) or wild type (+/+). Normal fetuses were usually littermates of the mutant fetuses. The ages of fetuses were determined by defining day 0 of pregnancy as that of the appearance of the vaginal plug. Pregnant mice were sacrificed by cervical dislocation, and the fetuses by decapitation. Mutant fetuses were recognized by the curvature of the spine and the absence of movement. The two half rib cages of each fetus were dissected in normal Krebs solution containing (in mM) 136 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES-NaOH; pH 7.4. The tissues were incubated at 37°C for approximately 10 min in phosphate buffer saline (Sigma Chemical Co., St. Louis, MO) containing collagenase (3 mg/ml, Type I; Sigma) and trypsin (1 mg/ml, Type III; Sigma). Myotubes were then mechanically dispersed and collected in plastic culture dishes (35 mm diameter) containing Krebs solution. Cells were maintained for approximately 2 h in Krebs solution before the experiments were performed. The bottom of the plastic dish was replaced by a thin glass coverslip for optical measurements.

Ca^{2+} current and charge movement

The standard patch-clamp technique was used in the whole-cell recording configuration. The external solution for current recording was (in mM) 130 TEA methanesulfonate, 10 $BaCl_2$ (or $CaCl_2$), 1 $MgCl_2$, 10^{-3} tetrodotoxin, 10 HEPES-TEA(OH); pH 7.4. The pipette solution consisted of (in mM) 140 Cs aspartate, 5 $MgCl_2$, 5 EGTA, 10 MOPS-CsOH pH 7.2. Standard patch electrodes were pulled from borosilicate glass and had tip resistance of 2–5 MΩ when they were filled with pipette solution. Recordings were made with an Axopatch 1D patch clamp and a headstage with a 50-MΩ feedback resistor (Axon Instruments, Foster City, CA). The recording chamber was mounted upon the stage of an inverted microscope for simultaneous measurement of cell fluorescence when appropriate. Junction potentials were nulled before seal formation. Seal resistance in cell-attached configuration varied from 0.5 to 5 GΩ. Cell capacitance was determined by integration of an uncompensated capacitive transient elicited by a 10-mV depolarizing pulse from a holding potential of −50 mV. Effective series resistance was estimated as described (Armstrong and Gilly, 1992) and was compensated up to the point of amplifier oscillation with the analog circuit provided by Axopatch. Three linear capacitive components and a leak component were canceled with a tunable analog circuit kindly provided by J. Vergara. Linearity of the analog compensation and pulse protocol in the range of −100 mV to +100 mV was verified with an RC cell model circuit. The data were filtered at 1–3 kHz and digitized at 50–100 μs. Data acquisition was performed with a TL1 DMA interface controlled by pCLAMP software (Axon Instrument). Current and charge movements from each cell were normalized to the uncompensated cell capacitance. All experiments were performed at room temperature.

For recordings of charge movement, the bath solution was supplemented with 0.5 mM $CdCl_2$ to block the ionic Ba^{2+} currents. Determination of the nifedipine-sensitive component of charge movement, Q_{ns}, required paired recordings in the same cell. To determine the stability of the cell, we repeated the same pulse protocol 3–10 times within a control period of 10–20 min. Reported Q_{ns}-V curves were from cells showing less than 10% variation in Q_{max} during the control period. Nifedipine was added to the bath solution in the range of 2–10 μM. In most cells, 2 μM nifedipine was sufficient to block Q_{ns}, and higher concentrations did not produce additional reduction in charge movement. However, 5–10 μM of nifedipine was routinely used. We implemented two pulse protocols to determine the Q-V relationship of a single cell. Protocol A consisted of a 25-ms test pulse P from holding potential −80 mV. To assist subtraction of the linear components, pulse P was preceded by four hyperpolarizing prepulses of amplitude $P/4$ ($-P/4$ pulses) with a duration of 25 ms and an interpulse period of 100 ms. An alternative protocol (protocol B) was designed to inactivate Na^+ and T-type Ca^{2+} channels and was therefore used to assess the amount of immobilization-resistant charge movement. Protocol B was similar to that used by Adams et al. (1990) and consisted of a step from holding potential −80 mV to −20 mV for 1 s, then to −50 mV for 5 ms, then to test potential P for 25 ms, then to −50 mV for 30 ms, and finally to the −80 mV holding potential. The $-P/4$ pulses of 25-ms duration and 500-ms interepisode duration followed this sequence from −80 mV. Table 1 shows Boltzmann parameters obtained from a fit of Q_{ON}-V curves in five cells subjected to both protocols. The estimated Q_{max}, $V_{1/2}$, and k with either protocol A or protocol B were not significantly different. Cell survival was longer with protocol A than with protocol B, presumably because of the smaller number of pulses delivered by protocol A. Because protocol A without $-P/4$ was used to record current in most instances, protocol A was used to compare charge movement in normal and mutant myotubes. Boltzmann parameters obtained from a fit of Q_{ON}-V or Q_{OFF}-V curves in 13 normal cells by use of protocol A are indicated in Table 1. Q_{ON} and Q_{OFF} were obtained by integration of ON and OFF currents, respectively. When necessary, a sloping baseline was fitted to the end of the pulse current and extrapolated to the edge of the voltage step. Q_{ON} and Q_{OFF} were similar (see Table 1) in cells in which Cd^{2+} effectively blocked tail Ca^{2+} current. When this was not the case, Q_{OFF} became contaminated, and $Q_{OFF} > Q_{ON}$. Contamination by tail current was rarely seen in mutant cells.

Intracellular Ca^{2+} transients

We measured intracellular Ca^{2+} by microfluorimetry of fura-2, using a dual alternating-excitation wavelength illuminator attached to an inverted microscope (Photon Technology International, London, Ontario, Canada). Cells were loaded with 0.1 mM fura-2 AM for 20 min at room temperature. The bath solution was the same for recording Ca^{2+} currents. The pipette solution was (in mM) 140 Cs aspartate, 5 $MgCl_2$, 0.1 EGTA, 10 MOPS-CsOH; pH 7.2. After a whole-cell recording configuration was established, a slit of ~40 × 70 μm was adjusted toward the center of the cell and away from the patch pipette. Fluorescence emission was separated from excitation by a dichroic cube with a 400-nm dichroic mirror and a 510/20 emission barrier filter. Emission ratios in response to alternate 340/380-nm excitation were taken at a rate of 200 ratios/s. The 340/380 fluorescence ratio at a given time during the Ca^{2+} transient was designated Rt and was

TABLE 1 Boltzmann parameters of charge movement in normal cells

Protocol	Q_{max} (nC/μF)	$V_{1/2}$ (mV)	k (mV)	n
Protocol A	7.85 ± 0.44	3.3 ± 4.0	16.2 ± 0.4	5
Protocol B	7.73 ± 0.38	3.0 ± 2.2	13.8 ± 0.6	5
Q_{ON}	7.04 ± 0.54	−1.0 ± 1.0	13.8 ± 0.8	13
Q_{OFF}	7.55 ± 0.63	−5.4 ± 2.2	11.6 ± 0.7	13

Protocols A (without prepulse) and B (with prepulse) were described in Materials and Methods and were performed in the same five normal cells. Q_{ON} and Q_{OFF} were obtained by integration of ON and OFF currents using Protocol A in a separate group of 13 normal cells. Q_{max}, $V_{1/2}$, and k (mean ± SE) correspond to averages of Boltzmann parameters fitted to each cell.

divided by the resting fluorescence ratio before the stimulus, Rt_0. Because the autofluorescence of cells was undetectable, the background fluorescence was measured in the same dish of dye-loaded cells from a field without cells. The background fluorescence was subtracted from the 340- and 380-nm fluorescence emission before the calculation of the emission ratio. In control recordings, the amplitude of the Ca^{2+} transient at the same voltage was reduced two- to threefold and the decay time constant decreased ninefold when the pipette solution contained 5 mM EGTA. The amplitude and decay of the Ca^{2+} transient were approximately the same when fura-2 (free acid) was loaded into the cell through the patch pipette at a concentration of 0.1 mM.

[^{3}H]PN 200–110 binding

Binding experiments were done as described by Pinçon-Raymond et al. (1985). Hind-limb muscles were dissected from normal and mutant mice. Tissues were frozen in liquid nitrogen and stored at −80°C before use. Muscles were thawed and immediately homogenized in ~2 ml of ice-cold 300 mM sucrose, 20 mM Tris-Cl; pH 7.4. Homogenates were filtered through four layers of moist cheesecloth, and the filtrate was immediately used for binding experiments. Aliquots of homogenates were incubated 45–60 min at 25°C in 100 μl of a 50 mM Tris-Cl, pH 7.4, containing 0.12–0.36 nM (+)-[5-methyl-^{3}H]-PN200-110 (85.8 Ci/mmol). The final concentration of the homogenate was 1.2–1.9 mg of protein/ml, determined by a Bradford assay (Bio-Rad, Richmond, CA) with bovine serum albumin used as a standard. Samples were filtered with a cell harvester on Whatman GF/C strip filters (Brandel, Gaithersburg, MD). Filters were washed twice with double glass-distilled water and were counted with 4 ml of scintillation fluid after 12 h. Nonspecific binding was measured in the presence of 10 μM of unlabeled nifedipine, which is a competitive antagonist of PN200-110. All experiments were made in duplicate, and counts were averaged.

Chemicals and abbreviations

Deionized glass-distilled water was used in all solutions. All salts were reagent grade. Nifedipine and Bay K 8644 were made as 10- and 5-mM stocks in absolute ethanol and stored in light-resistant containers. Nifedipine and TTX (tetrodotoxin) were obtained from Sigma Chemical Co. (St. Louis, MO). Bay K8644 was from Calbiochem (La Jolla, CA). Fura-2 AM was from Molecular Probes (Eugene, OR). (+)-[5-methyl-^{3}H]-PN200-110 (85.8 Ci/mmol) was from New England Nuclear Research Products (Boston, MA). EGTA is ethyleneglycol-bis-(*b*-aminoethyl ether) *N,N,N′,N′*-tetra acetic acid; HEPES is *N*-2-hydroxyethyl piperazine-*N′*-2-ethanesulfonic acid; MOPS is 3-[*N*-morpholino]propane-sulfonic acid; TEA is tetraethylammoniun.

Curve fitting

Curve fitting was done with the standard Marquardt–Levenberg algorithm provided by either Sigmaplot (Jandel, San Rafael, CA) or pClamp (Axon Instruments). The relationship between charge movement (Q) or L-type current conductance (G) and pulse potential was fitted by a Boltzmann equation expressed as follows: $A = A_{max}/(1 + \exp(-(V - V_{1/2})/k))$. A_{max} is the maximum amount of movable charge (Q_{max}) or the maximum conductance (G_{max}), $V_{1/2}$ is the potential at which $A = A_{max}/2$, and k is the slope factor. To determine the cell-to-cell variability, we fitted a Boltzmann curve for each cell separately. The mean ± SE of averages of Boltzmann parameters fitted separately to each cell are shown in Tables 1 and 2 and the text. In some cases we made a Boltzmann fit to the population average Q-V curve obtained by pooling data from many cells (see the solid curves in Figs. 6 and 7). The time constant τ_1 describing activation of Ca^{2+} current was obtained from a fit of pulse current at each voltage according to $I(t) = K[(1 - \exp(-t/\tau_1)\exp(-t/\tau_2)$, where K is constant and τ_2 describes inactivation. All averages are presented as mean ± SE.

RESULTS

Fig. 1A shows two distinct Ca^{2+} channel types in freshly dissociated intercostal myotubes from normal fetal 18-day-old mice. The charge carrier was 10 mM Ba^{2+}, but other recordings described below were made in 10 mM Ca^{2+}. Test pulses of 300 ms from a holding potential of −80 mV revealed a low-voltage-activated transient component with a time to peak of ~20 ms at −10 mV and a high-voltage-activated sustained component. Previous studies identified the transient component as a T-type current and the sustained component as a DHP-sensitive L-type current (Cognard et al., 1986; Beam and Knudson, 1988). We further tested for the presence of these two components by analyzing the voltage dependence of current measured either at the peak or at the end of the 300-ms test pulse (Fig. 1 *B*). The lowest voltage that evoked a T-type current was −40 mV, whereas that for the L-type current was −10 mV. Similar measurements for β_1-null myotubes are shown in Fig. 1 *C* and *D*. In the mutant myotube, the density of sustained current (hereafter called $I_{\beta null}$) was much smaller than the density of L-type current of normal cells (see below). In

TABLE 2 Boltzmann parameters of charge movement and Ba^{2+} conductance in normal and β_1 null skeletal muscle cells

	Normal				Mutant				
Parameters	Q_{max} (nC/μF)	$V_{1/2}$ (mV)	k (mV)	n	Q_{max} (nC/μF)	$V_{1/2}$ (mV)	k (mV)	n	Mutant/Normal
Q_{total}	6.94 ± 0.61	−1.50 ± 1.13	13.19 ± 0.66	19	2.54 ± 0.20	−9.32 ± 1.75	8.75 ± 0.62	21	0.37
Q_{ns}	2.84 ± 0.86	−2.24 ± 2.18	9.74 ± 1.05	6	1.14 ± 0.24	−12.88 ± 6.62	4.30 ± 1.65	5	0.40
Normalized Q_{ns}	–	−2.96	9.46	6	–	−15.65	8.52	5	–
	G_{max}(pS/pF)				G_{max}(pS/pF)				
G_{Ba}	161.61 ± 8.93	−0.20 ± 0.83	3.96 ± 0.30	18	17.88 ± 1.34	4.88 ± 1.31	6.48 ± 0.43	17	0.11
Normalized G_{Ba}	–	−0.62	4.29	18	–	4.38	6.72	17	–
Q_{ns}/Q_{total}	0.41	–	–	–	0.45	–	–	–	–
G_{Ba}/Q_{ns}	56.90	–	–	–	15.68	–	–	–	–

Q_{max}, $V_{1/2}$, and k (mean ± SE) of Q_{total}, Q_{ns}, and G_{Ba} correspond to averages of Boltzmann parameters fitted to the Q-V and G-V curves of each cell. $V_{1/2}$ and k of normalized Q_{ns} and G_{Ba} correspond to Boltzmann parameters fitted to the average Q/Q_{max}-V and G/G_{max}-V curves constructed by pooling data from all cells.

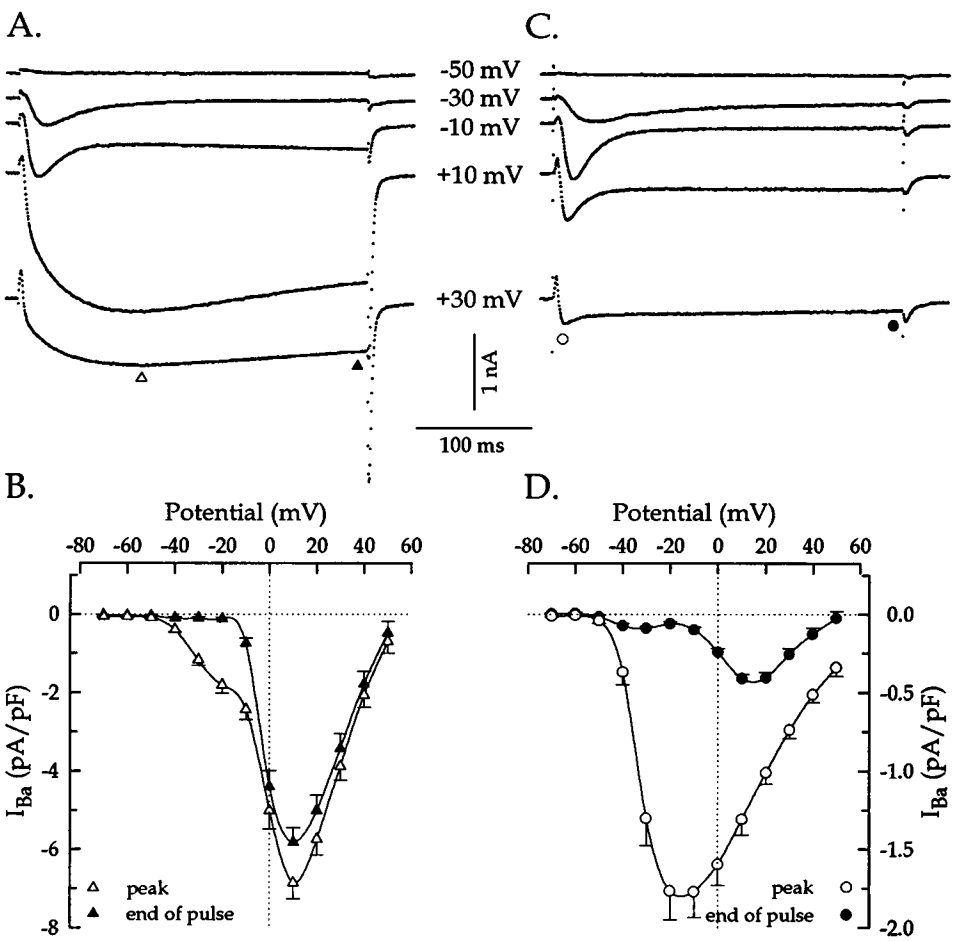

FIGURE 1 Ba^{2+} currents recorded in normal (*A*) and mutant (*C*) myotubes. Voltage-clamp records during 300-ms depolarizing steps from holding potential −80 mV to the indicated potential. The capacitance of normal and mutant myotubes was 336 and 432 pF, respectively. Voltage dependence of the average Ba^{2+} current density measured at the end of the pulse (●, ▲) and at the peak (○, △) in normal (*B*; n = 18) and mutant (*D*; n = 17) myotubes.

contrast, the T-type current was, on the average, approximately equal in density to the T-type current of normal cells. The peak T-type current in response to a test pulse to −20 mV from holding potential −80 mV was 1.81 ± 0.21 pA/pF in normal (n = 18) and 1.76 ± 0.18 pA/pF in mutant (n = 17) cells. Both L-type and T-type currents were invariably present in 94 normal and 92 mutant cells analyzed.

A separation of T-type and L-type currents was possible by changing the holding potential from −80 to −40 mV, as shown in Fig. 2*A* and *C*, for which 10 mM Ca^{2+} was used as the charge carrier. The −40-mV holding potential eliminated the T-type current of normal and mutant cells, but the normal L-type (Fig. 2 *A*) or $I_{\beta null}$ (Fig. 2 *C*) components were unchanged. *I-V* curves measured at the end of the 300-ms test pulse from the two holding potentials are shown in Fig. 2 *B* and *D* for normal and mutant cells, respectively. In both populations there was a small amount of T-type current that remained not inactivated at the end of the pulse when the holding potential was −80 mV but not when the holding potential was −40 mV. However, the *I-V* curves were invariant with holding potential when the test potential was positive. This indicated that there was little if any contamination by the T-type Ca^{2+} current in this voltage range. Based on these considerations, the normal L-type Ca^{2+} current density at +20 mV was 5.20 ± 0.38 (n = 14), and $I_{\beta null}$ was 0.41 ± 0.04 pA/pF (n = 12). Fig. 3 shows that the G_{Ca}-V curve for the $I_{\beta null}$ current was shifted toward positive potentials (~5.5 mV at the midpoint) and was less steep than that for the L-type current of normal cells. The same phenomenon was observed when 10 mM Ba^{2+} was used as the charge carrier (see Table 2). The decrease in current amplitude and the positive shift of the *G-V* curve were similar to observations made in heterologous expression systems when the α_{1S} subunit was expressed in the absence of β_1 (Lory et al, 1992). However, the magnitude of the shift in β_1-null cells is uncertain because $V_{1/2}$ varied with the extrapolated Ca^{2+} current reversal potential.

To establish the relationship between $I_{\beta null}$ and the normal L-type current, we determined the sensitivity of $I_{\beta null}$ to nifedipine and Bay K 8644. Fig. 4A shows Ba^{2+} current at a test potential that activated both T-type and $I_{\beta null}$ currents in a mutant cell, during a control run and after a bath application of 5 μM nifedipine. Currents from a separate mutant cell during a control run and after bath application of 5 μM Bay K 8644 are shown in Fig. 4 *C*. The inhibitory effect of nifedipine and the stimulatory effect of Bay K 8644 were much more pronounced on the current at the end of the pulse than on the peak current dominated by the T-type current. *I-V* curves for end-of-pulse current are shown before and after bath application of nifedipine (Fig.

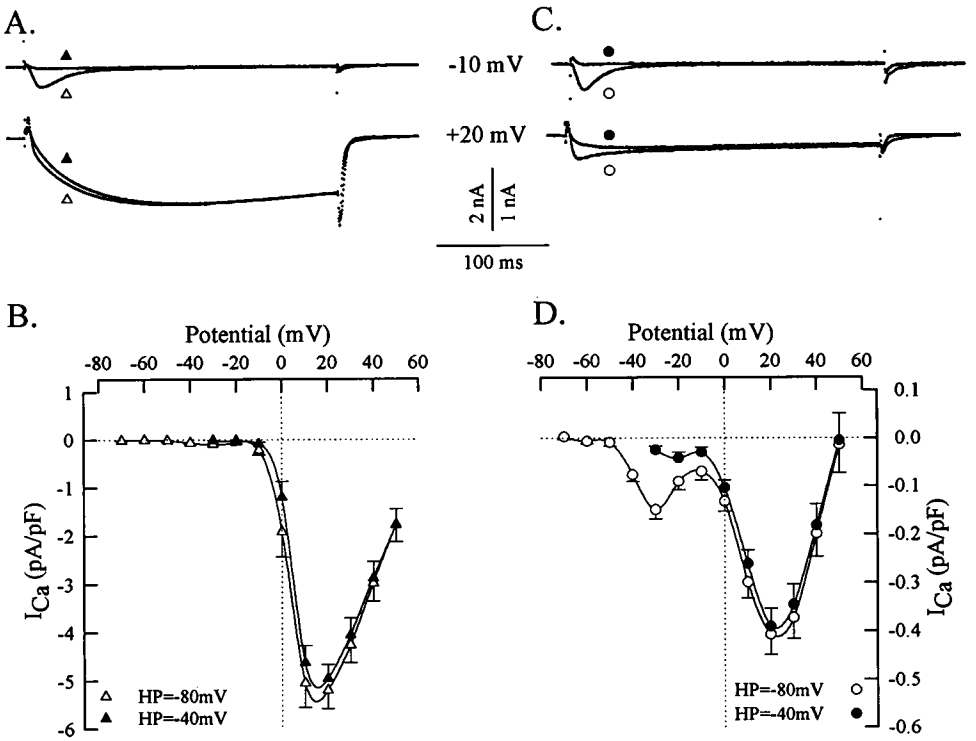

FIGURE 2 Ca^{2+} currents in normal (*A*) and mutant (*C*) myotubes. Voltage-clamp records during 300-ms depolarizing steps from holding potential −80 mV (○, △) or −40 mV (●, ▲) to the indicated potential. The capacitance of normal and mutant myotubes was 417 and 489 pF, respectively. Voltage dependence of the average Ca^{2+} current density measured at the end of the pulse from a holding of potential of −80 mV (○, △) or −40 mV (●, ▲) in normal (*B*; n = 14) and mutant (*D*; n = 12) myotubes.

4 *B*) or Bay K 8644 (Fig. 4 *D*). $I_{\beta null}$ at + 20 mV was reduced to background levels by nifedipine, whereas the T-type current at −30 mV was unchanged. In contrast, Bay K 8644 increased $I_{\beta null}$ ~2.5-fold with no effect on the T-type current. These results indicated that $I_{\beta null}$ is sensitive to DHPs and therefore is an L-type current. Moreover, the increase in $I_{\beta null}$ current by Bay K 8644 was more pronounced than that observed in the normal L-type current at the same drug concentration (~1.3-fold, not shown). The higher sensitivity of $I_{\beta null}$ to Bay K 8644 is consistent with observations in heterologous expression systems when the α_{1S} subunit is expressed in the absence of β_1 (Varadi et al., 1991; Lory et al., 1992).

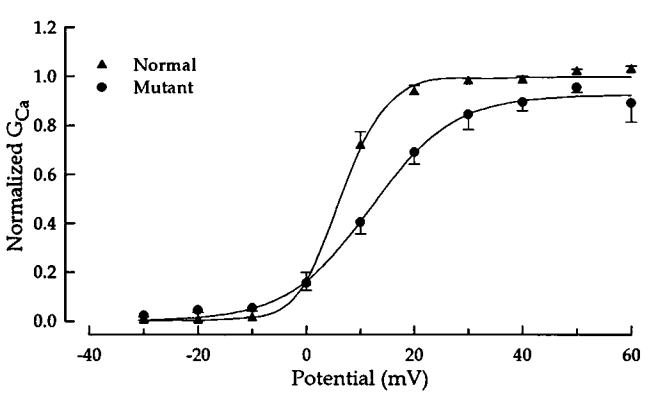

FIGURE 3 Voltage dependence of the normalized Ca^{2+} conductance measured from holding potential −40 mV in normal (▲, n = 14) and mutant (●, n = 12) myotubes. Values of Boltzmann parameters of the fit shown by the curves are $V_{1/2}$ = 6.43 mV and $V_{1/2}$ =11.83 mV; k = 3.95 mV and k = 7.63 mV, for normal and mutant, respectively.

The effect of DHPs, the reduced current density, and the positive shift of the *I-V* curve suggested that $I_{\beta null}$ could originate from a dihrodropyridine complex lacking β_1. The presence of β_1 either increases (Lacerda et al., 1991; Singer et al., 1991; Varadi et al., 1991; Wei et al., 1991; Lory et al., 1992), decreases (Perez-Garcia et al., 1995) or has no effect on (Itagaki et al., 1992; Lory et al., 1993) the activation rate of the α_1 current, depending on the expression system and α_1 subtype. Therefore, we compared the kinetics of $I_{\beta null}$ and normal L-type currents. Fig. 5 *A* shows scaled traces of normal L-type (triangles) and $I_{\beta null}$ (circles) Ca^{2+} currents activated by a test pulse to +10 mV. A pulse duration of 750 ms and a holding potential of −40 mV were necessary for observation of L-type current inactivation and elimination of the T-type current. $I_{\beta null}$ activated faster and inactivated more slowly than the L-type current of normal cells. The solid curves in Fig. 5 *A* correspond to a fit using an equation derived from a linear kinetic scheme with a single activation and inactivation step (see Materials and Methods). This equation adequately fitted the pulse current over a wide range of test potentials. Fig. 5 *B* shows time constants for activation of normal L-type and $I_{\beta null}$ currents obtained by a fit of traces at each test potential. $I_{\beta null}$ activated in a voltage-dependent manner, and the activation time constant was significantly smaller than that of the L-type current of normal cells over a wide range of positive test potentials. This result was consistent with the observation in heterologous expression systems showing that β_1

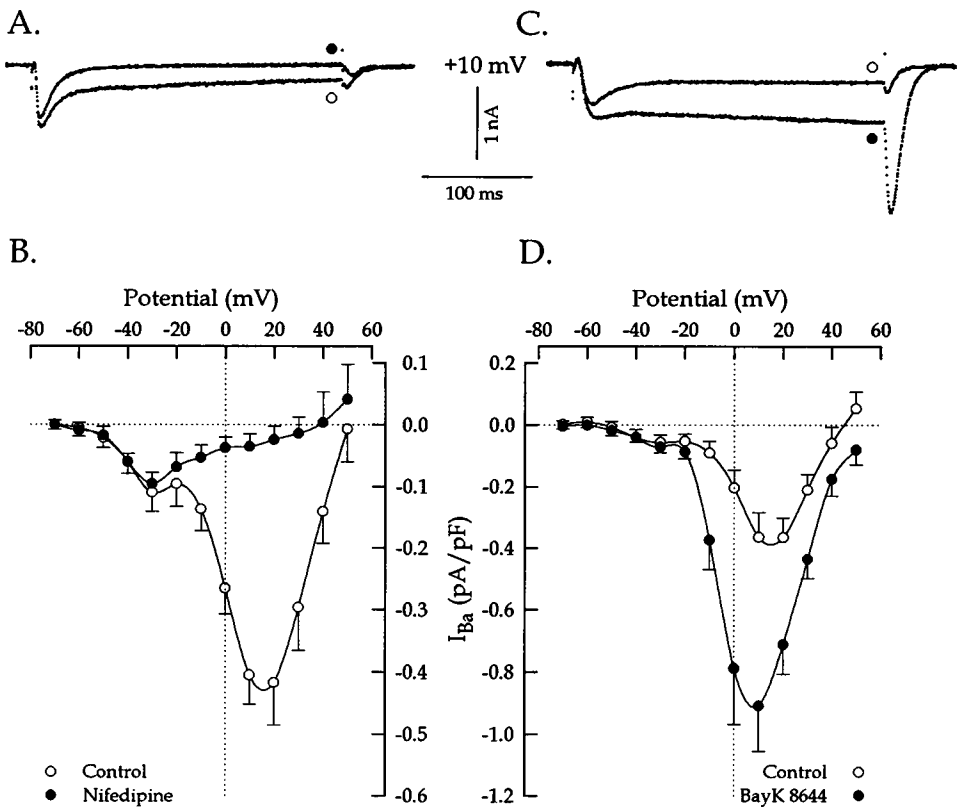

FIGURE 4 Ba^{2+} currents in response to a 300-ms depolarizing pulse from holding potential −80 mV to +10 mV in control (○) and 10 min after addition of 5 μM (●) nifedipine (*A*) or 5 μM Bay K 8644 (*C*). The capacitance of the myotubes was 614 and 590 pF in *A* and *C*, respectively. Voltage dependence of the average Ba^{2+} current density measured at the end of the pulse in control and 10 min after addition of nifedipine (*B*; $n = 9$) or Bay K 8644 (*D*; $n = 7$).

slowed the activation of the Ca^{2+} current produced by α_{1C} subunit in HEK cells (Perez-Garcia et al., 1995) and disagreed with other studies showing that β_1 accelerated the kinetics of α_{1C} subunit in oocytes (Singer et al., 1991; Wei et al., 1991) and of α_{1S} in L cells (Lacerda et al., 1991; Varadi et al., 1991; Lory et al., 1992).

Fig. 6 shows recordings of nonlinear charge movement in a normal and a mutant myotube of similar cell capacitance obtained with protocol A described in Materials and Methods. At −40 mV and more negative test potentials, charge movement was not detectable. With test potentials more positive than −40 mV, ON and OFF components of charge movement became progressively larger, and they saturated at test potentials more positive than +30 mV. Some records showed a small nonlinear leak component, which presumably was due to residual outward current (see Bean and Rios, 1989). The most obvious feature of charge movement in the mutant cells was the reduction in magnitude (Fig. 6 *B*). After they were normalized for uncompensated cell capacitance, the *Q-V* curves of 19 normal and 21 mutant cells were pooled. Fig. 6 *C* shows that the population average *Q-V* curve could be adequately fitted by a single Boltzmann equation with parameters decribed in the figure legend for both groups. In normal cells the fitted Q_{max} agreed well with previous reports in either freshly dissociated (Beam and Knudson, 1988; Strube et al., 1992) or cultured (Garcia et al., 1994) myotubes. The Q_{max} of mutant cells was approximately 2.8-fold lower than that of normal cells. The threshold for movement of the first charges occurred at approximately the same potential in both preparations, but saturation occurred earlier in mutant cells. Consequently, the *Q-V* curve of mutant myotubes was shifted ~8 mV toward negative potentials relative to the *Q-V* curve of normal myotubes. Averages of Boltzmann parameters obtained by fitting each cell separately are shown in Table 2. A paired *t*-test of the average Q_{max}, $V_{1/2}$, and k (normal versus mutant) indicated that the difference was significant ($p < 0.001$) for all parameters.

Because protocol A may result in charge movements from several different channel types, we determined the amount of nifedipine-sensitive charges in both preparations. Previous studies indicated that the DHP-sensitive component of charge movement originates from the voltage sensor of E-C coupling and from gating currents of L-type Ca^{2+} channels (Rios and Brum, 1987; Pizarro et al., 1988; Adams et al., 1990). To determine the amount of nifedipine-sensitive charges, we subjected the same cell during the control period to a minimum of three runs of eleven test pulses from −50 to +50 mV and later to a minimum three runs following bath application of 2–10 μM nifedipine (Fig. 7 *A* and *B*). The nifedipine-sensitive component was obtained by subtraction at each test potential in each cell, and the average is shown in Fig. 7 *C*. Boltzmann parameters fitted to the population average Q_{ns}-*V* curves are given in the figure legend. Similar to the Q_{total}-*V* curve, the Q_{ns}-*V* curve of mutant cells had a reduced Q_{max}, and there was a significant shift in $V_{1/2}$ toward negative potentials. These differences were also apparent in Table 2, corresponding to averages of

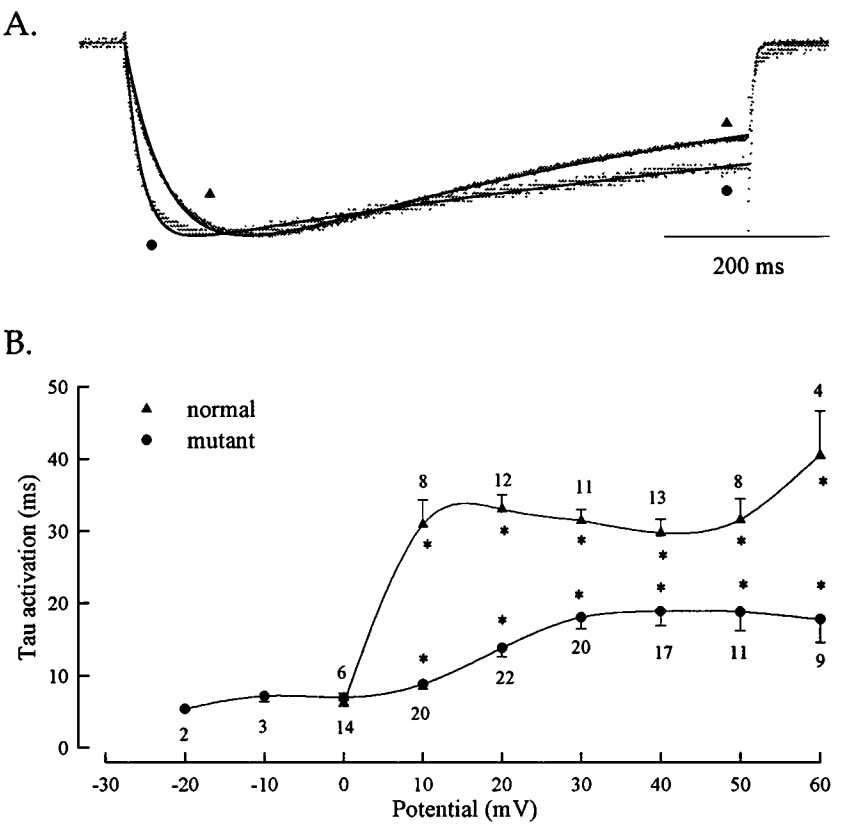

FIGURE 5 (*A*) Scaled traces of Ca^{2+} current from a normal (▲) and a mutant (●) myotube in response to a 750-ms depolarizing pulse from a holding potential of −40 mV to +10 mV. The solid curves correspond to a fit using the biexponential equation for $I(t)$ described in Materials and Methods with $K = -1.29$ pA, $\tau_1 = 54.7$ ms, $\tau_2 = 750$ ms for normal and $K = -1.07$ pA, $\tau_1 = 19.9$ ms, $\tau_2 = 1416$ ms for mutant. (*B*) Time constant for current activation for normal (▲) and mutant (●) myotubes. Numbers near symbols indicate the number of cells. Asterisks indicate time constants of normal and mutant cells found to be significantly different to $p < 0.005$ according to a paired *t*-test.

Boltzmann parameters fitted separately to each cell. Table 2 shows that the proportion of nifedipine-sensitive charge movement relative to the total charge movement was approximately the same in normal and mutant cells ($Q_{ns(max)}/Q_{total(max)} \sim 0.40$). Thus, charge movements appeared to be equally sensitive to nifedipine in both groups.

A lower density of Q_{ns} in mutant myotubes could originate from a lower density of DHP receptors in the cell membrane. To determine whether this was the case, we performed radioligand binding analysis in homogenates of normal and mutant skeletal muscles, using the high-affinity DHP [^{3}H]PN 200–110. In all cases (Table 3) we observed a saturable and specific binding of the radio label in a range of concentration from 0.12 to 36 nM. Scatchard representations resulted in a straight line from which B_{max} and K_d were estimated. In mutant muscle, [^{3}H]PN 200–110 maximum binding site density (B_{max}) was reduced ~3.9-fold, and the dissociation constant (Kd) was ~3.6-fold higher. The decrease in B_{max} in the β_1-null muscle agreed with results in heterologous systems (Lacerda et al., 1991; Varadi et al., 1991; Wei et al., 1991; Lory et al., 1992; Nishimura et al., 1993; Perez-Garcia et al., 1995). The decrease in DHP binding site density in the homogenate of mutant muscle agreed with the decrease in Q_{ns}, thus confirming a lower density of membrane-bound DHP receptors.

We previously found that intracellular Ca^{2+} transients could not be elicited by action potentials in β_1-null cells (Gregg et al., 1996). We therefore determined the relationship between the L-type Ca^{2+} currents and the elicited Ca^{2+} transients. A test pulse of variable amplitude and a duration of 50 ms was applied to normal cells. The same pulse with a duration of 500 ms was applied to mutant cells to compensate for the fact that $I_{\beta null}$ had a much lower density. A holding potential of −40 mV was used in all cases. Between subsequent pulses there was a resting period of 40 s. In the normal cell (Fig. 8A) the onset of the Ca^{2+} transient was instantaneous with the onset of the test pulse. The peak fluorescence was followed by a decay that had a $t_{1/2}$ of 0.3–0.5 s, consistent with previous reports (Grouselle et al., 1991; Garcia and Beam, 1994; Strube et al., 1994). The Ca^{2+} current and the Ca^{2+} transient activated over the same range of pulse potential. However, at depolarizations more positive than +20 mV the peak of the Ca^{2+} transient remained constant, whereas the Ca^{2+} current decreased as the test pulse approached the reversal potential. The constancy of the Ca^{2+} transient, in the face of a decreasing Ca^{2+} current, indicated that a voltage-dependent rather than a Ca^{2+} entry mechanism participated in the generation of Ca^{2+} transients, in agreement with previous observations (Cognard et al., 1992, 1993; Garcia et al., 1994; Strube et al., 1994). Caffeine induced a Ca^{2+} transient much larger than that induced by cell depolarization (not shown). Therefore, the constancy of the transient at positive potentials did not reflect dye saturation. Unlike in normal cells, Ca^{2+} transients could not be elicited in mutant cells at any test potential (Fig. 8 *C*). Fig. 9 shows the voltage dependence of the peak of the Ca^{2+} transient, and this is compared with the voltage dependence of the integral of the Ca^{2+} current in the

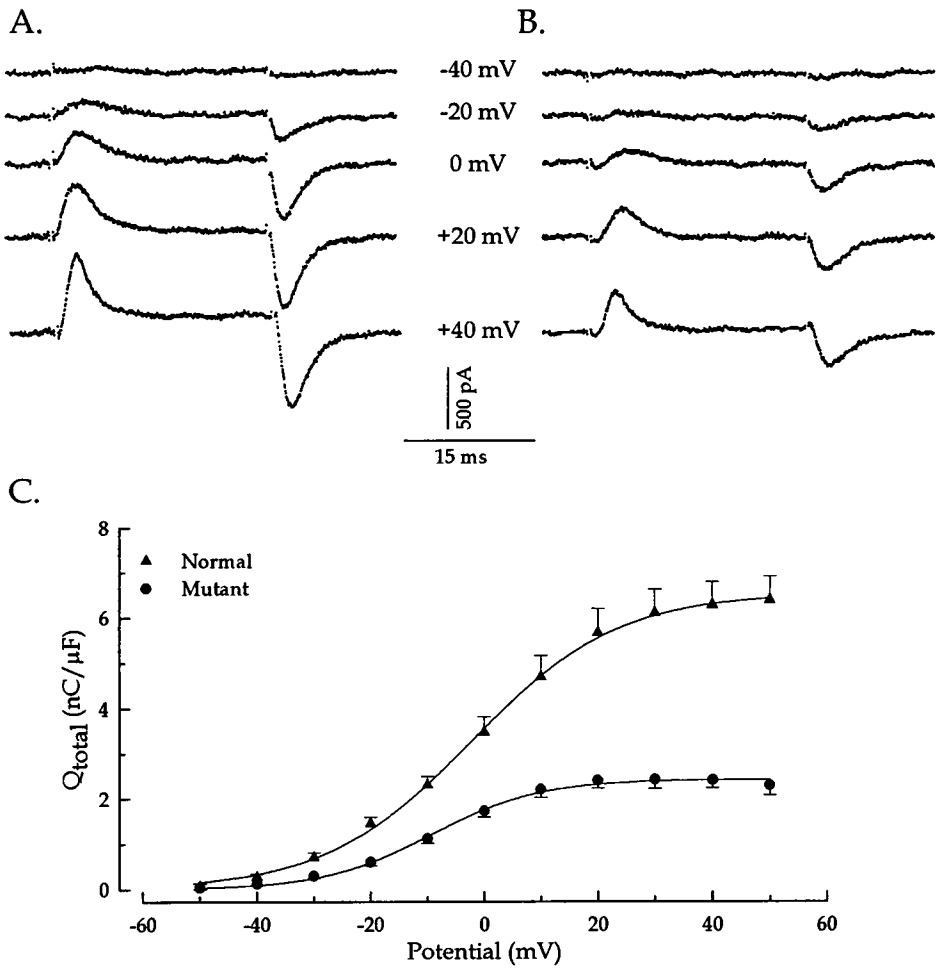

FIGURE 6 Asymmetric currents produced by intramembrane charge movements in a normal (*A*) and a mutant (*B*) myotube. Voltage-clamp traces were recorded during 25-ms depolarizing steps from holding potential −80 mV to the indicated potentials. The capacitance of normal and mutant myotubes was 321 and 382 pF, respectively. (*C*) *Q-V* curves of normal (▲, n = 19) and mutant (●, n = 21) myotubes. Curves correspond to a Boltzmann fit of the mean with parameters Q_{max} = 6.55 and Q_{max} = 2.43 nC/μF; $V_{1/2}$ = −2.33 and $V_{1/2}$ = −9.25 mV; k = 12.85 and k = 9.49 mV for normal and mutant, respectively.

same cells. In agreement with previous results in myotubes in culture (Garcia et al., 1994), the amplitude of the Ca^{2+} transients saturated at potentials more positive than +40 mV. A Boltzmann relationship fitted to the Ca^{2+} transient is shown by the curve and resulted in a fitted Rt/Rt_0(max) = 2.36, $V_{1/2}$ = 2.1 mV, and k = 8.6 mV. The $V_{1/2}$ and k were within the range reported for normal myotubes in culture (Garcia and Beam, 1994; Garcia et al., 1994). In the case of mutant cells, there was a much larger entry of Ca^{2+} into the cell because of the longer pulse duration. However, Ca^{2+} entry did not trigger a Ca^{2+} transient.

Because the absence of a Ca^{2+} transient in β_1-null cells could be due to insufficient storage of Ca^{2+} in the sarcoplasmic reticulum, we performed intracellular Ca^{2+} measurements in the presence of caffeine (Fig. 10). Bath application of 5 mM caffeine to a mutant cell resulted in a robust Ca^{2+} transient that lasted for several seconds. Furthermore, preapplication of 1 μM thapsigargin for 10 min eliminated the caffeine-induced Ca^{2+} transient. These data indicated that caffeine caused a release of Ca^{2+} from a thapsigargin-sensitive store, presumably the sarcoplasmic reticulum, and that the Ca^{2+} storage and release capacities of mutant cells were not impaired.

DISCUSSION

β_1-null cells fail to contract in response to electrical stimulation despite the presence of a normal action potential and a caffeine-induced contracture, suggesting that E-C coupling is impaired (Gregg et al., 1996). In the present study we investigated the events that are known to control excitation–Ca^{2+} release from normal skeletal muscle cells and that might lead to the failure of Ca^{2+} release in mutant cells. The significant findings of this study are the following: 1) The presence of an L-type current in mutant cells identified by its high voltage threshold, slow inactivation, and sensitivity to DHP agonists and antagonists. The L-type current density of mutant cells is reduced ~13-fold compared with normal; 2) The presence of charge movements in mutant cells that are sensitive to nifedipine. The density of Q_{ns} of mutant cells is reduced ~2.5-fold, and the $V_{1/2}$ of the Q_{ns}-V curve is ~10 mV more negative than that of normal cells; 3) The ~3.9-fold reduction in the maximum number of [^{3}H]PN200-110 binding sites in homogenates of mutant skeletal muscle; 4) The absence of a depolarization-induced Ca^{2+} transient in mutant cells despite the presence of thapsigargin- and caffeine-sensitive Ca^{2+} storage and release

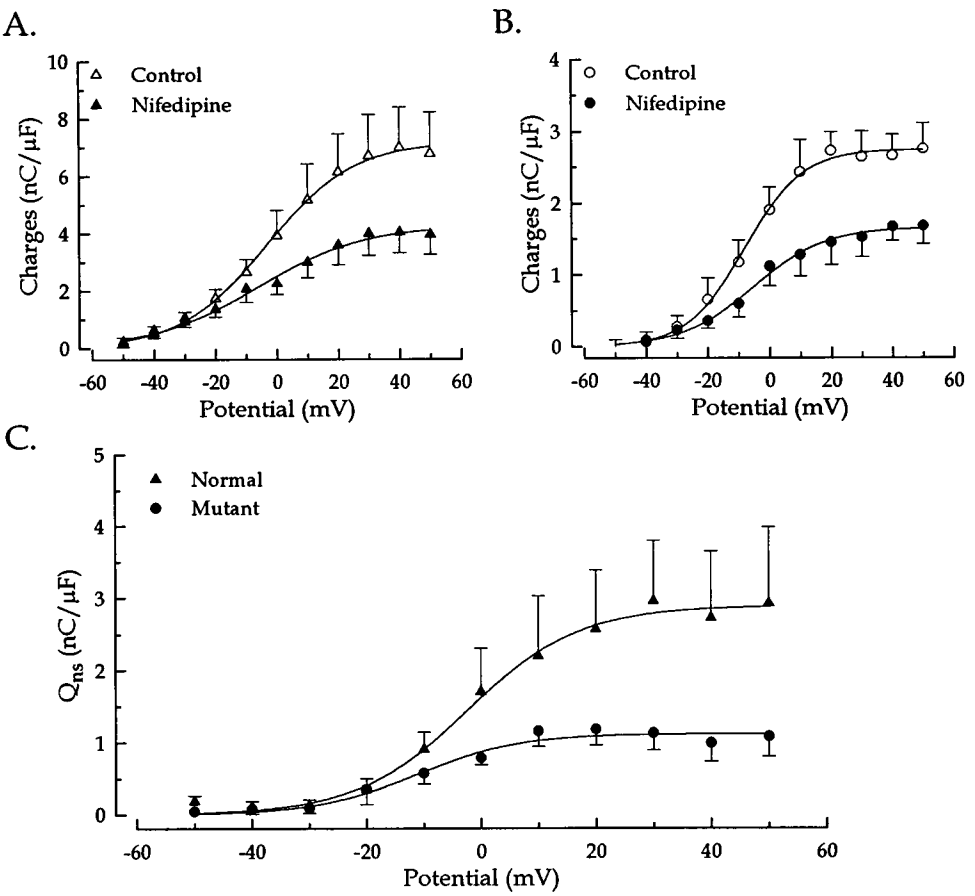

FIGURE 7 Q-V curves of normal (A; n = 6) and mutant (B; n = 5) myotubes during control (○, △) and 5 min after addition of nifedipine (●, ▲). (C) Q_{ns}-V curves of normal (▲, n = 6) and mutant (●, n = 5) myotubes. For each cell, Q_{ns} was calculated by subtracting the value of Q obtained after addition of nifedipine from the value of Q obtained in control at each voltage. Curves correspond to a Boltzmann fit of the mean with parameters Q_{max} = 2.89 and Q_{max} = 1.12 nC/μF; $V_{1/2}$ = −2.43 and $V_{1/2}$ = −11.25 mV; k = 9.7 and k = 8.72 mV for normal and mutant, respectively.

capacities. Taken together, these observations suggest that mutant cells have a low density of functional voltage sensors and that these voltage sensors are unable to support E-C coupling.

TABLE 3 Binding parameters of [^{3}H]PN200-110 to skeletal muscle homogenates from normal and mutant mice

B_{max} (fmol/mg)	K_d (nM)	n
	Normal	
18	0.19	5
19	0.42	5
24	0.53	5
46	0.67	5
Mean ± SE		
27 ± 6	0.45 ± 0.10	–
	Mutant	
5	1.72	8
5	1.59	8
11	1.51	8
Mean ± SE		
7 ± 2	1.61 ± 0.06	–

Leg muscles from the indicated number of fetuses were pooled and homogenized as described in Materials and Methods. Binding parameters B_{max} and K_d were obtained from a straight line fitted to a Scatchard representation of specific [^{3}H]PN 200-110 binding in the concentration range of 0.12–36 nM. Parameters from separated experiments were averaged.

The fact that $I_{\beta null}$ differs from the normal L-type Ca^{2+} current not only in current density but also in kinetics and other properties raises the question of the origin of $I_{\beta null}$. The slower inactivation, higher DHP sensitivity, and positive shift of the I-V curve suggest that $I_{\beta null}$ may be a down-regulated current produced by $\alpha_{1S}\gamma\alpha_2/\delta$ complexes, as occurs when α_1 is expressed in the absence of β_1 in heterologous systems (Singer et al., 1991; Varadi et al., 1991; Lory et al., 1992, 1993). However, $I_{\beta null}$ could be unrelated to α_{1S}, because studies in α_{1S}-deficient dysgenic myotubes describe a Ca^{2+} current, I_{dys}, that shares similarities to $I_{\beta null}$ (Adams and Beam, 1989). For example, $I_{\beta null}$ and I_{dys} have a similar peak current density, which in 10 mM Ca^{2+} amounts to 0.61 ± 0.08 pA/pF for I_{dys} (Adams and Beam, 1989) and to 0.41 ± 0.04 for $I_{\beta null}$. An internal control for this comparison is provided by the peak T-type current, which has identical density in either dysgenic or β_1-null myotubes (1.62 ± 0.17 pA/pF for dysgenic cells and 1.76 ± 0.18 pA/pF for β_1-null cells). Furthermore, $I_{\beta null}$, like I_{dys}, is more sensitive to Bay K 8644 and activates faster than the normal L-type current. However, there are significant differences between $I_{\beta null}$ and I_{dys} reflected in the I-V curves. I_{dys} is shifted to the left of the I-V curve of normal cells, whereas $I_{\beta null}$ is slightly right shifted. This is a rather significant difference, implying that the mechanisms of voltage-dependent activation of I_{dys} and $I_{\beta null}$ are

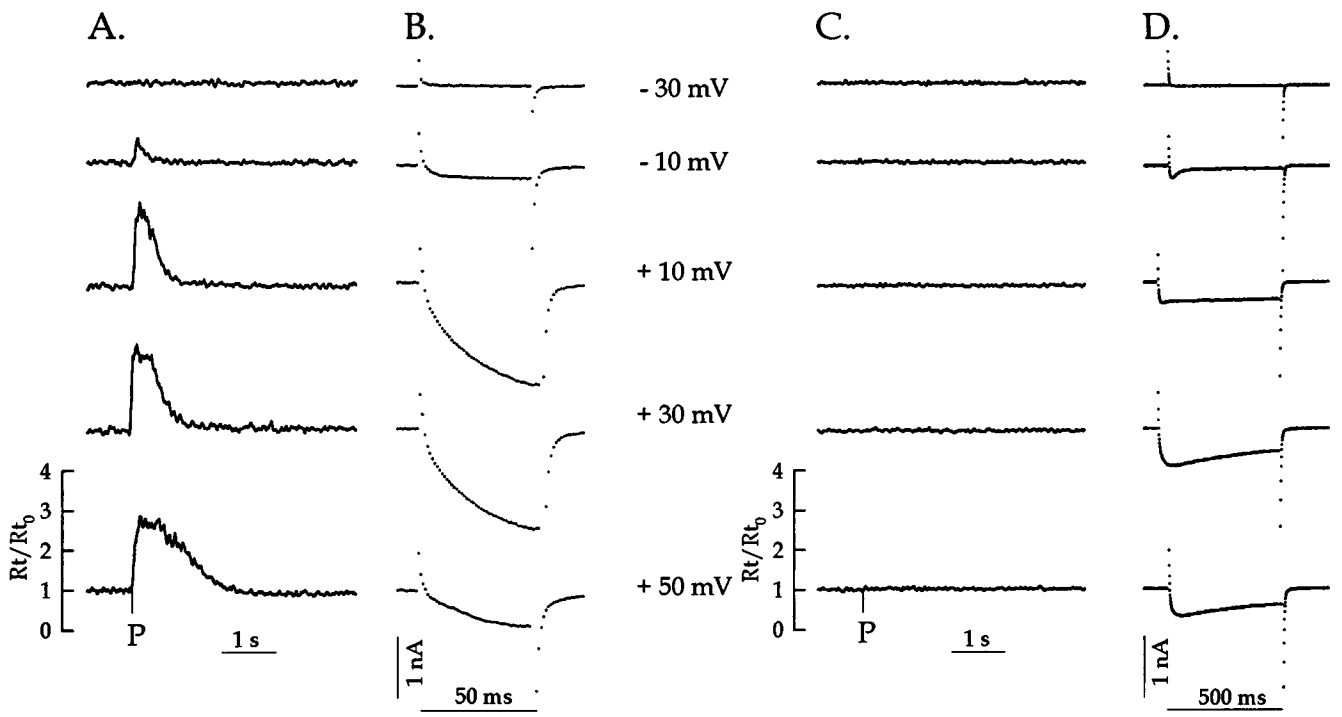

FIGURE 8 Simultaneous recordings of Ca^{2+} transients and Ca^{2+} currents under voltage clamp in a normal (*A*, *B*) and a mutant (*C*, *D*) myotube. Ca^{2+} transients (*A*, *C*) and Ca^{2+} currents (*B*, *D*) were elicited by a test pulse from holding potential of −40 mV to the indicated potential. The test pulse duration was 50 ms in normal and 500 ms in mutant. The capacitance of normal and mutant myotubes was 473 and 490 pF, respectively. *P* indicates the onset of the test pulse.

different and may represent different arrangements of Ca^{2+} channel subunits. A comparison of $I_{\beta null}$ and Ca^{2+} currents obtained by overexpression of α_{1S} in β_1-null cells should establish whether $I_{\beta null}$, like I_{dys}, represents an embryonic current unrelated to skeletal-type DHP complexes or whether $I_{\beta null}$ is the functional expression of an $\alpha_{1S}\gamma\alpha_2/\delta$ complex.

The interpretation of charge movements was facilitated by the observation that Q_{max} was resistant to immobilization by a prepulse and thus unlikely to originate from either Na^+ channels or T-type Ca^{2+} channels (Table 1). Protocol B included a long prepulse to −20 mV and a 5-ms repolarization to −50 mV before the test pulse. In Ca^{2+} recording solutions containing 10 mM Na^+, the Na^+ current and the T-type Ca^{2+} current inactivated completely during the prepulse to −20 mV. During the test pulse, more than 70% of I_{Na+} remained inactivated. Evidently, there was some recovery of I_{Na+} during the 5-ms repolarization to −50 mV. However, the repolarizing period could not be made shorter, as doing so also prevented recovery of nifedipine-sensitive charges moved during the prepulse. In contrast, the T-type current remained inactivated during the test pulse of protocol B. If Q_{max} obtained by protocol A were severely contaminated by Na^+ channel gating current, Q_{ON} of protocol A should have been larger than Q_{ON} of protocol B because of charge immobilization during protocol B. This was not observed (see Table 1), and therefore protocol A was sufficient to detect the bulk of the immobilization-resistant component of charge movements in these cells. The fact that protocol A and protocol B detected similar amounts of nonlinear charge movements agrees with the conclusions reached that in adult skeletal muscle Na^+-channel density is low and therefore that the contribution to detectable charge movements is minor (Almers, 1978). Further, a comparison of charge movements in fetal cardiac cells by use of protocols with and without prepulses did not detect a significant immobilization-sensitive component (Field et al., 1988). We are not aware of a similar comparison for fetal skeletal muscle besides the data provided here. However, skeletal myotubes in culture do have a significant amount of immobilization-sensitive component (Adams et al., 1990).

The $Q_{total(max)}$ of normal cells measured here (6.9 ± 0.61 nC/μF) agreed well with measurements in acutely dissociated 1–5-day-old rat myotubes (6.0 ± 1.7 nC/μF; Beam and Knudson, 1988); acutely dissociated 18-day-old dysgenic heterozygous *(+/mdg)* fetal mouse myotubes (6.3 ± 1.4 nC/μF; Shimahara et al., 1992); and 7–11-day-old mouse myotubes in culture (5.1 ± 0.9 nC/μF; Garcia et al., 1994). On the other hand, $Q_{total(max)}$ of β_1-null cells (2.54 ± 0.2 nC/μF) was slightly larger than that of acutely dissociated 18-day-old dysgenic homozygous *(mdg/mdg)* fetal mouse myotubes (1.7 ± 0.3 nC/μF; Shimahara et al., 1992) or approximately equal to that of *mdg/mdg* myotubes in culture (2.5 ± 0.8 nC/μF; Adams et al., 1990) when the $Q_{total(max)}$ of cultures of normal cells run in parallel was 7.9 ± 1.4 nC/μF (Adams et al., 1990). Based on the numerical simi-

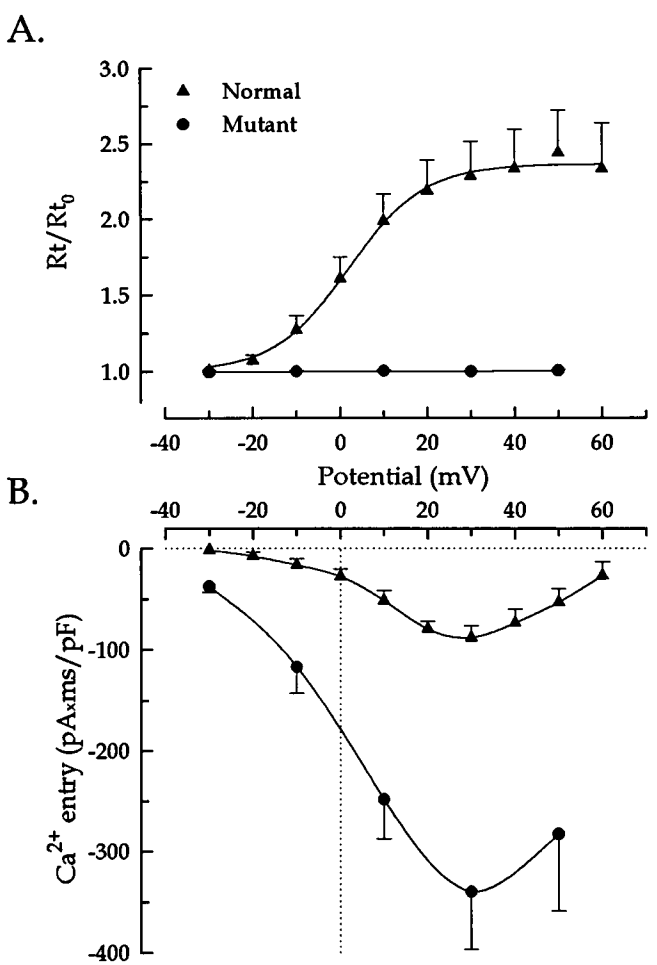

FIGURE 9 Ca^{2+} currents and Ca^{2+} transients recorded in normal (▲, n = 13) and mutant (●, n = 8) myotubes. (*A*) The peak amplitude of the Ca^{2+} transient is plotted as a function of the test potential. Test pulses were applied from −40 mV and had a duration of 50 ms in normal cells and 500 ms in mutant cells. The sigmoidal curve corresponds to a Boltzmann fit of the mean fluorescence ratio of normal cells with parameters Rt/Rt_0(max) = 2.36 and $V_{1/2}$ = 2.1 mV; k = 8.6 mV. (*B*) Integral of Ca^{2+} current recorded during the Ca^{2+} transient in the same cells.

larity between the charge movements of dysgenic and β_1-null myotubes, it could be proposed that $Q_{\text{total(max)}}$ may not be sufficient to support a voltage-dependent transduction mechanism of E-C coupling in the β_1-null cell. We believe that this explanation is unlikely because measurements in acutely dissociated normal 14-day-old fetal myotubes indicate that $Q_{\text{total(max)}}$ or $Q_{\text{ns(max)}}$ is the same as or smaller than these parameters in β_1-null cells, yet 100% of the 14-day-old cells can elicit Ca^{2+} transients in response to a depolarization and ~50% of these responses are independent of Ca^{2+} influx (Strube at al., 1992, 1994). Thus the reduction of charge movement per se, to the extent observed in β_1-null cells, cannot explain the absence of a voltage-activated Ca^{2+} transient. It is conceivable that some inherent property of charge movement may be different in mutant cells, and this may account for the uncoupling. This would be the case if Q_{ns} of β_1-null cells originated from charge movement of either a developmental form of α_1 such as the reported cardiac isoform (Chaudhari and Beam, 1993) or the two-motif isoform (Malouf et al., 1992) or from the presence of I_{dys} but not of α_{1S}, which is the isoform that specifically confers skeletal type E-C coupling to the developing skeletal muscle cell (Tanabe et al., 1990b). In this respect, it should be mentioned that Q_{ns} has been reported in cultures of dysgenic myotubes (B. A. Adams, personal communication) but not in freshly dissociated cells (Shimahara et al., 1990, 1992). Although the present results could not identify the source of Q_{ns} in β_1-null cells, they do not exclude the possibility that Q_{ns} may originate from $\alpha_{1S}\gamma\alpha_2/\delta$ complexes present in the plasma membrane in amounts much lower than in normal cells and that these complexes without β_1 fail to initiate E-C coupling.

The reduction in [^{3}H]PN200-110 binding to microsomes of β_1-null muscle and the reduction in Q_{ns} suggest that the density of functional α_{1S} subunits or $\alpha_{1S}\gamma\alpha_2/\delta$ complexes in the surface or transverse tubular membranes of mutant cells is reduced. A reduction in plasma membrane α_{1S} agrees with the observation that expression of β_1 in L cells or β_{2a} in HEK 293 cells increases the amount of immunodetectable α_{1S} or α_{1C}, respectively, located in the plasma membrane of transfected cells (Krizanova et al., 1995; Chien et al., 1995). Also, a reduction in the density of α_{1C} subunits expressed in the membrane of HEK 292 cells when α_{1C} is expressed in absence of β_{1a} or β_3 has been deduced from the decrease in the density of charge movements of the expressed Ca^{2+} channels (Josephson and Varadi, 1996; Kamp et al., 1996). However, with regard to the observed ~13-fold decrease in Ca^{2+} current of mutant cells, the reduction in plasma membrane α_{1S} may account only for a ~2.5-to-3.9-fold reduction in L-type Ca^{2+} current, because these are the extent of the reduction of $Q_{\text{ns(max)}}$ and B_{max} in these cells. The additional ~5.2-to-3.3-fold reduction in L-type Ca^{2+} current must thus be explained as being due to different mechanisms. We have considered the possibility that β_1 may affect the *G-V* and *Q-V* curves of the skeletal DHP receptor differently. Previous studies suggest that intermolecular coupling between charge movements and opening of the pore is facilitated by the β subunit because the $G_{\text{max}}/Q_{\text{max}}$ ratio in much higher in cells expressing α_{1C} and β_{2a} or α_{1C} and β_{1a} than in cells expressing α_{1C} alone (Neely et al., 1993; Kamp et al., 1996). Inasmuch as $G_{\text{Ba}}/Q_{\text{ns}}$ was much larger in normal than in mutant cells (Table 2), $I_{\beta\text{null}}$ could display less intramolecular coupling than could the normal L-type current. Thus, at the same voltage, opening of $I_{\beta\text{null}}$ channels would be less probable than the opening of normal L-type channels. This possibility can be investigated directly by cell-attached patch-clamp recording or by fusion of transverse tubular membranes to planar bilayers (Affolter and Coronado, 1985). Also, the β subunit could be critically involved in the arrangements of DHP receptors into arrays of tetrads in the transverse tubular membrane. When this arrangement is disrupted, the L-type Ca^{2+} current is drastically reduced, but charge movements are almost normal (Takekura et al., 1995; Nakai et al., 1996). Finally, it must

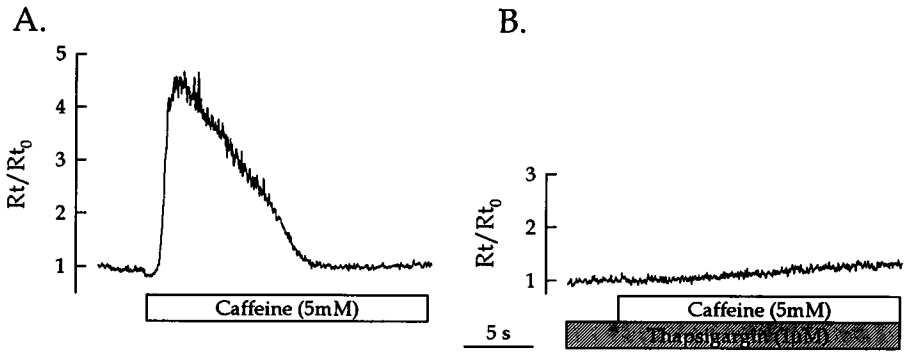

FIGURE 10 Ca^{2+} transient induced in a mutant myotube by 5 mM caffeine in the absence (*A*) and presence (*B*) of 1 μM thapsigargin.

be emphasized that in muscle cells the interpretation of the *G/Q* ratio may not be so straightforward as in heterologous expression systems. It has been argued that in skeletal muscle the α_1 isoform that generates the L-type Ca^{2+} current is functionally different from the isoform that initiates E-C coupling (Pizarro et al., 1988; De Jongh et al., 1989; Bois et al., 1991). If this is ultimately the case, measurements of *G* and *Q* in muscle cells may not reflect properties of a single population of DHP receptors with the same functional properties. The role of β subunits in modulating DHP receptor function as well as the number of α_1 subunits in the membrane can now be elucidated in the homologous expression system provided by the β_1-null skeletal muscle cells.

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