Introduction

In skeletal muscle, entry of external divalent cations occurs in response to sustained weak depolarizations or repetitive electrical stimulation via a process which has been termed Excitation-Coupled Ca\(^{2+}\) Entry (ECCE), which is hypothesized to require the interaction of the ryanodine receptor (RyR1), the L-type Ca\(^{2+}\) channel (DHPR) and another unidentified cation channel. Thus, ECCE is absent in myotubes lacking either the DHPR (dysgenic) or RyR1 (dyspedic). Furthermore, ECCE, as measured by Mn\(^{2+}\) quench of Fura-2, is reconstituted by expression of a mutant DHPR \(\alpha_{1S}\) subunit (SkEIIIK) thought to be impermeable to divalent cations. Previously, we showed that the bulk of depolarization-induced Ca\(^{2+}\) entry could be explained by the skeletal L-type current. Accordingly, one would predict that any Ca\(^{2+}\) current similar to the endogenous current would restore such entry and that this entry would not require coupling to either the DHPR or RyR1. Here, we show that expression of the cardiac \(\alpha_{1C}\) subunit in either dysgenic or dyspedic myotubes does result in Ca\(^{2+}\) entry similar to that ascribed to ECCE. We also demonstrate that, when potentiated by strong depolarization and Bay K 8644, SkEIIIK supports entry of Mn\(^{2+}\). These results strongly support the idea that the L-type channel is the major route of Ca\(^{2+}\) entry in response to repetitive or prolonged depolarization of skeletal muscle.

The cardiac \(\alpha_{1C}\) subunit can support excitation-triggered Ca\(^{2+}\) entry in dysgenic and dyspedic myotubes

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Abbreviations: DHPR, 1,4-dihydropyridine receptor; EC, excitation-contraction; ECCE, excitation-coupled Ca\(^{2+}\) entry; RyR, ryanodine-sensitive intracellular Ca\(^{2+}\) release channel; SOCE, store-operated Ca\(^{2+}\) entry; SR, sarcoplasmic reticulum; TRP, transient receptor potential

Depolarization-induced entry of divalent ions into skeletal muscle has been attributed to a process termed Excitation-Coupled Ca\(^{2+}\) Entry (ECCE), which is hypothesized to require the interaction of the ryanodine receptor (RyR1), the L-type Ca\(^{2+}\) channel (DHPR) and another unidentified cation channel. Thus, ECCE is absent in myotubes lacking either the DHPR (dysgenic) or RyR1 (dyspedic). Furthermore, ECCE, as measured by Mn\(^{2+}\) quench of Fura-2, is reconstituted by expression of a mutant DHPR \(\alpha_{1S}\) subunit (SkEIIIK) thought to be impermeable to divalent cations. Previously, we showed that the bulk of depolarization-induced Ca\(^{2+}\) entry could be explained by the skeletal L-type current. Accordingly, one would predict that any Ca\(^{2+}\) current similar to the endogenous current would restore such entry and that this entry would not require coupling to either the DHPR or RyR1. Here, we show that expression of the cardiac \(\alpha_{1C}\) subunit in either dysgenic or dyspedic myotubes does result in Ca\(^{2+}\) entry similar to that ascribed to ECCE. We also demonstrate that, when potentiated by strong depolarization and Bay K 8644, SkEIIIK supports entry of Mn\(^{2+}\). These results strongly support the idea that the L-type channel is the major route of Ca\(^{2+}\) entry in response to repetitive or prolonged depolarization of skeletal muscle.
like Ca\(^{2+}\) entry can indeed be reconstituted by expression of the cardiac DHPR \(\alpha_{1C}\) subunit in either dysgenic or dyspedic myotubes. Thus, neither expression of the DHPR \(\alpha_{1S}\) subunit nor the presence of RyR1 is required for the ECCE-like Ca\(^{2+}\) entry observed after expression of \(\alpha_{1C}\). In addition, we show that SkEIIIK can conduct Mn\(^{2+}\) when potentiated in by the strong depolarization in the presence of the L-type channel agonist ±Bay K 8644, consistent with the possibility that the Mn\(^{2+}\) quench observed previously in SkEIIIK-expressing dysgenic cells may have resulted from Mn\(^{2+}\) entry via the mutant channel itself.

| Table 1. Activation and decay of \(\alpha_{1C}\)-mediated L-type current during extended depolarizations |
|---------------------------------------------------------------|
| Test potential | \(I_{\text{peak}}\) (pA/pF) | \(t_{\text{peak}}\) (s) | \(t_{\text{0.5 decay}}\) (s) | \(r_9\) |
|-----------------|-------------------|-----------------|-----------------|-------|
| -20 mV | -2.5 ± 0.4 (5) | 1.57 ± 1.20 | ND | ND |
| -10 mV | -5.2 ± 0.3 (5) | 0.044 ± 0.010 | 6.31 ± 1.46 | 0.48 ± 0.03 |
| 0 mV | -6.1 ± 0.8 (5) | 0.027 ± 0.005 | 5.06 ± 0.84 | 0.35 ± 0.02 |

Currents were recorded from dysgenic myotubes expressing \(\alpha_{1C}\)-CFP. Data presented in Table 1 were obtained with 9.8 s test potentials to -20, -10 mV and 0 mV, from a holding potential of -80 mV, with 2 mM Ca\(^{2+}\) in the external recording solution. \(r_9\) is ratio of the current remaining at 9 s to the peak current. ND: decay kinetics at 20 mV were not quantified because the current failed to decay to ½ peak amplitude during the 9.8 s test pulse.

Results

L-type Ca\(^{2+}\) currents mediated by cardiac DHPR \(\alpha_{1C}\) subunits inactivate slowly and conduct substantial current at weak test potentials in physiological Ca\(^{2+}\). When expressed in dysgenic myotubes, cardiac DHPR \(\alpha_{1C}\) subunits produce robust L-type Ca\(^{2+}\) current. Since our previous results indicated that ECCE could be largely attributed to Ca\(^{2+}\) influx via native skeletal muscle L-type Ca\(^{2+}\) channels, we reasoned that \(\alpha_{1C}\) would also be capable of supporting ECCE-like Ca\(^{2+}\) entry. In the presence of 2 mM external Ca\(^{2+}\), ECCE can be strongly evoked by prolonged exposure to 60 or 80 mM K\(^{+}\); these ionic conditions produce membrane potentials in the range of -15 to 0 mV (Allen PD and López JR, personal communication). To determine whether \(\alpha_{1C}\)-CFP-mediated L-type current can be observed this range of potentials, we recorded currents in 2 mM external Ca\(^{2+}\) in response to 200 ms test pulses. As shown in Figure 1A and B, L-type currents mediated by \(\alpha_{1C}\)-CFP began to activate at -20 mV, and produced substantial L-type current at -10 and 0 mV in 2 mM external Ca\(^{2+}\) (n = 6). Since ECCE is normally evoked by exposures to elevated K\(^{+}\) on the order of seconds, we also measured currents in response to 9.8 s depolarizations to -20, -10 and 0 mV in 2 mM external Ca\(^{2+}\). As shown in Figure 1C, steps to -10 and 0 mV yielded L-type current which peaked quickly, inactivated modestly within ~1 s and then was relatively well maintained (Table 1). Taken together, the data in Figure 1A–C indicate that long, weak depolarizations elicit substantial, sustained \(\alpha_{1C}\)-CFP-mediated L-type current under conditions similar to those used to evoke ECCE.

ECCE-like Ca\(^{2+}\) entry is observed in dysgenic myotubes expressing cardiac \(\alpha_{1C}\) subunits. In order to assess whether the slowly inactivating L-type current mediated by \(\alpha_{1C}\)-CFP could support Ca\(^{2+}\) influx similar to that attributed to ECCE, we applied 60 mM K\(^{+}\) to dysgenic myotubes expressing \(\alpha_{1C}\)-CFP after block of SR Ca\(^{2+}\) release by ryiodine pre-treatment (200 μM, >1 hr, 37°C). Upon depolarization with 60 mM K\(^{+}\), dysgenic myotubes expressing \(\alpha_{1C}\)-CFP showed a large Ca\(^{2+}\) entry (n = 9; Fig. 2A). In control experiments, no quantifiable Ca\(^{2+}\) transients were observed in CFP-negative dysgenic cells (n = 18; Fig. 2B). Consistent with the idea that the Ca\(^{2+}\) entry observed
in cells expressing $\alpha_{1C}$-CFP resulted from L-type current, 10 $\mu$M nifedipine caused a large reduction in the rate of rise of depolarization-induced Ca$^{2+}$ transients and a decrease in the peak $\Delta F/F$ (n = 7; Fig. 2C and E). 50 $\mu$M nifedipine completely eliminated the transients (n = 2; Fig. 2D). Thus, the block of Ca$^{2+}$ entry via $\alpha_{1C}$ channels expressed in dysgenic skeletal myotubes by nifedipine (summarized in Fig. 2E) was comparable to the block of native cardiac L-type current by nifedipine in canine ventricular myocytes with a holding potential of -80 mV.$^{19}$

ECCE-like Ca$^{2+}$ entry is observed in dysgenic myotubes expressing cardiac $\alpha_{1C}$ subunits. The presence of ECCE-like Ca$^{2+}$ entry in dysgenic myotubes expressing $\alpha_{1C}$-CFP suggests that conformational coupling to RyR1 is not essential for ECCE, because $\alpha_{1C}$ does not engage in interactions with RyR1 that produce skeletal-type EC coupling.$^{10-12,16-18,20,21}$ To test the requirement for RyR1 more directly, we examined whether Ca$^{2+}$ transients could be evoked in dysgenic myotubes expressing $\alpha_{1C}$-CFP. In 7 of 10 dysgenic myotubes displaying cyan fluorescence, we observed a large Ca$^{2+}$ entry in response to 60 mM K$^+$ ($AF/F = 1.45 \pm 0.21; n = 7$; Fig. 3A). It is unlikely that the observed transients in dysgenic myotubes transfected with $\alpha_{1C}$-CFP arose from RyR3-mediated Ca$^{2+}$ release from the SR because the myotubes were pretreated with 200 $\mu$M ryanodine for 1 hour (37°C) prior to experiments.

Because dysgenic myotubes do express relatively unaltered levels of the native skeletal DHPR,$^{23}$ it is possible that these endogenous DHPRs contributed to the Ca$^{2+}$ entry observed in dysgenic myotubes transfected with $\alpha_{1C}$-CFP. However, only small Ca$^{2+}$ currents are produced by the endogenous DHPRs in dysgenic myotubes.$^{14,23-28}$ Additionally, in agreement with previous studies,$^{12,4,7}$ no elevated K$^+$-evoked Ca$^{2+}$ transients were observed in untransfected dysgenic cells (n = 15; Fig. 3B). Furthermore, no measurable Ca$^{2+}$ entry was observed in dysgenic myotubes treated with 5 $\mu$M $\pm$Bay K 8644 ($\tau_{deact} = 4.2 \pm 0.5$ vs. 1.3 $\pm$0.4 ms, respectively; n = 4; $p < 0.006$, t-test; Fig. 4C). The slow rate of decay of this $\pm$Bay K 8644-sensitive inward current in ±Bay K 8644 relative to control ($\tau_{deact} = 4.2 \pm 0.5$ vs. 1.3 $\pm$0.4 ms, respectively; n = 4; $p < 0.006$, t-test; Fig. 4C) indicates that it is an inward ionic tail current carried by Mn$^{2+}$.
entry when we expressed $\alpha_{1C}$ in dysgenic myotubes, which lack RyR1. Thus, L-type current is sufficient to produce ECCE-like Ca$^{2+}$ entry without an absolute requirement for $\alpha_{1S}$ or RyR1. In addition to the L-type channel, skeletal muscle is known to express other cation channels (e.g., TRPs) which could contribute to Ca$^{2+}$ entry.30 Likewise, divalent cations may also enter the myoplasm via an unidentified non-electrogenic exchanger. 31 However, one would expect that ECCE-like depolarization-induced Ca$^{2+}$ entry in normal myotubes would be largely attributable to the skeletal L-type current since this current is of similar magnitude to the L-type current in dysgenic myotubes expressing $\alpha_{1C}$.

The Ca$^{2+}$ entry in dysgenic myotubes expressing $\alpha_{1C}$ clearly does not depend on the conformational state of RyR1, as previously proposed for ECCE,1 since $\alpha_{1C}$ cannot engage in either orthograde10-12,14,16-18,20 or retrograde14,21 conformational coupling with RyR1. The robust Ca$^{2+}$ entry observed in dysgenic myotubes expressing $\alpha_{1C}$ was not attributable to endogenous $\alpha_{1S}$ since we never saw such entry in naïve dysgenic myotubes. The absence of ECCE-like Ca$^{2+}$ entry in dysgenic myotubes can be explained by the loss of retrograde coupling between RyR1 and the skeletal muscle DHPR, without which the L-type current has very small amplitude.14,23-28 Moreover, we did not detect depolarization-induced Ca$^{2+}$ entry when we expressed $\alpha_{1C}$ in dysgenic myotubes, which lack RyR1. Thus, L-type current is sufficient to produce ECCE-like Ca$^{2+}$ entry without an absolute requirement for $\alpha_{1S}$ or RyR1. In addition to the L-type channel, skeletal muscle is known to express other cation channels (e.g., TRPs) which could contribute to Ca$^{2+}$ entry.30 Likewise, divalent cations may also enter the myoplasm via an unidentified non-electrogenic exchanger. 31 However, one would expect that ECCE-like depolarization-induced Ca$^{2+}$ entry in normal myotubes would be largely attributable to the skeletal L-type current since this current is of similar magnitude to the L-type current in dysgenic myotubes expressing $\alpha_{1C}$.

In the current study, we demonstrated that the cardiac DHPR $\alpha_{1C}$ subunit could produce sustained L-type Ca$^{2+}$ current in response to weak depolarizations when expressed in dysgenic myotubes (Fig. 1). Furthermore, depolarization of either dysgenic or dyspedic myotubes expressing $\alpha_{1C}$ caused entry of extracellular Ca$^{2+}$ (Figs. 2 and 3, respectively) resembling that previously attributed to ECCE in normal myotubes.1-7 In addition, we also showed that SkEIIIK, a mutant DHPR $\alpha_{1S}$ subunit thought not to be permeable to divalent cations,8 will conduct Mn$^{2+}$ in a potentiated state induced by ±Bay K 8444 and strong depolarization (Fig. 4). This Mn$^{2+}$ permeability may account for the depolarization-induced Mn$^{2+}$ entry previously described in response to depolarization of dysgenic myotubes expressing SkEIIIK.1 Together, these results provide further support for the view that the L-type Ca$^{2+}$ current is the molecular basis of the Ca$^{2+}$ entry, which has been attributed to ECCE.

ECCE-like entry of Ca$^{2+}$ has been described as requiring the presence of both the DHPR $\alpha_{1S}$ subunit and of RyR1. However, we have now shown that Ca$^{2+}$ entry resembling ECCE occurs after expression of $\alpha_{1C}$ in dysgenic myotubes, which lack $\alpha_{1S}$. Furthermore, we observed similar depolarization-induced Ca$^{2+}$ entry when we expressed $\alpha_{1C}$ in dysgenic myotubes, which lack RyR1. Thus, L-type current is sufficient to produce ECCE-like Ca$^{2+}$ entry without an absolute requirement for $\alpha_{1S}$ or RyR1. In addition to the L-type channel, skeletal muscle is known to express other cation channels (e.g., TRPs) which could contribute to Ca$^{2+}$ entry.30 Likewise, divalent cations may also enter the myoplasm via an unidentified non-electrogenic exchanger.31 However, one would expect that ECCE-like depolarization-induced Ca$^{2+}$ entry in normal myotubes would be largely attributable to the skeletal L-type current since this current is of similar magnitude to the L-type current in dysgenic myotubes expressing $\alpha_{1C}$.
induced Ca\(^{2+}\) entry in dysgenic myotubes even after treatment with the dihydropyridine agonist \(\pm\)Bay K 8644 (Fig. 3C). The inability of \(\pm\)Bay K 8644 to potentiate the natively-expressed DHPRs of dysgenic myotubes to the point of producing ECCE is not surprising in this regard because Bay K 8644 only weakly potentiates L-type current in dysgenic myotubes.\(^{25}\) In particular, Bay K 8644 only targets the fast, transient activation phase of the L-type current leaving the slow, sustained component of the current unaffected.\(^{25}\)

If ECCE actually represents divergent cation influx via L-type channels, then the question arises as to why ECCE was observed in dysgenic myotubes expressing SkEIIIK, as assayed by Mn\(^{2+}\) quench of Fura-2 dye.\(^1\) In Figure 4, the slowly decaying tail currents produced by SkEIIIK indicate that potentiated SkEIIIK channels can conduct Mn\(^{2+}\). In addition to producing slowly decaying tail currents after potentiation by \(\pm\)Bay K 8644 and strong depolarization (Fig. 4B and C), SkEIIIK channels which entered into the potentiated state during prolonged step depolarizations would also be expected to produce inward Mn\(^{2+}\) current and this might account for the Mn\(^{2+}\) quench observed by Cherednichenko et al. in dysgenic myotubes expressing SkEIIIK.\(^1\) It should be noted that we have not been able to detect such inward Mn\(^{2+}\) currents during step depolarizations (data not shown). However, even during prolonged depolarization, only a fraction of all channels would be converted to the potentiated state.\(^{32}\) Thus, any inward current through the potentiated SkEIIIK channels would be obscured by outward current through non-potentiated channels. Whether or not this explanation is correct, our present results indicate that skeletal L-type current alone is sufficient to account for the bulk of the voltage-dependent Ca\(^{2+}\) entry observed in ryanodine-treated normal myotubes\(^4\) and that this large entry would make it very difficult to detect any additional entry that might occur via a pathway other than the L-type channel.

In the present study, we have provided additional support for our hypothesis that the skeletal muscle L-type Ca\(^{2+}\) current is the molecular basis of ECCE.\(^4\) Since ECCE has been shown to help maintain myoplasmic Ca\(^{2+}\) levels during repetitive electrical stimuli,\(^{1,6}\) it now appears that the L-type Ca\(^{2+}\) current contributes to this essential function and may also be partially responsible for SR Ca\(^{2+}\) store refilling during activity. Furthermore, previous work has shown that mutations of RyR1 that result in malignant hyperthermia appear to accentuate ECCE and that this altered behavior of ECCE could contribute to triggering episodes of this pharmacogenetic muscle disease.\(^{3,5,33}\) Thus, an important goal for future research will be to define the involvement of the skeletal L-type Ca\(^{2+}\) current in malignant hyperthermia.

**Materials and Methods**

**Myotube culture and cDNA expression.** All procedures involving mice were approved by the University of Colorado-Denver Institutional Animal Care and Use Committee. Primary cultures of dysgenic (mdg/mdg) or dysgenic (Ryr1/Ryr1) myotubes were prepared as described previously.\(^{34}\) Cultures were grown for 6–7 days in a humidified 37°C incubator with 5% CO\(_2\) in Dulbecco’s Modified Eagle Medium (DMEM; #15-017-CM, Mediatech, Herndon, VA), supplemented with 10% fetal bovine serum/10% horse serum (Hyclone Laboratories, Logan, UT). This medium was then replaced with differentiation medium (DMEM supplemented with 2% horse serum). For electrophysiology, dysgenic myotubes were microinjected with a solution of either \(\alpha\)C-ECFP (200 ng/μl)\(^{35}\) and pEYFP-C1 (10 ng/μl; Clontech, Palo Alto, CA), or SkEIIIK (400 ng/μl)\(^4\) and pEYFP-C1 (10 ng/μl). For Ca\(^{2+}\) imaging experiments, single nuclei of either dysgenic or dysgenic myotubes were microinjected with a solution of cDNA encoding \(\alpha\)C-ECFP (200 ng/μl). Fluorescent myotubes were used in experiments two days following microinjection.

**Measurement of ionic currents.** Pipettes were fabricated from borosilicate glass and had resistances of ~1.5 MΩ when filled with internal solution, which consisted of (mM): 140 Cs-aspartate, 10 Cs\(_2\)-EGTA, 5 MgCl\(_2\) and 10 HEPES, pH 7.4 with CsOH. The external solution contained (mM): 145 tetraethylammonium-Cl, 2 CaCl\(_2\) or 10 mM MnCl\(_2\), 0.003 tetrodotoxin and 10 HEPES, pH 7.4 with tetraethylammonium-OH. For generation of current-voltage (I-V) relationships, linear capacitative and leakage currents were determined by averaging the currents elicited by eleven, 30-mV hyperpolarizing pulses from the holding potential of -80 mV. Test currents were corrected for linear components of leak and capacitive current by digital scaling and subtraction of this average control current. In all other experiments, -P/4 subtraction was employed. Electronic compensation was used to reduce the effective series resistance (usually to <1 MΩ) and the time constant for charging the linear cell capacitance (usually to <0.5 ms). L-type currents were filtered at 2 kHz and digitized at 5–10 kHz. In most cases, a 1-s prepulse to -20 mV followed by a 50-ms repolarization to -50 mV was administered before the test pulse\(^{36}\) to inactivate T-type Ca\(^{2+}\) channels. Cell capacitance was determined by integration of a transient from -80 mV to -70 mV using Clampex 8.0 and was used to normalize current amplitudes (pA/pF). I-V curves were fitted using the following equation:

\[
I = G_{\infty} \times [(V - V_{nrev})/(1 + \exp[-(V - V_{1/2})/k_c])],
\]

(Eq. 1)

where \(I\) is the peak current for the test potential \(V\), \(V_{nrev}\) is the current reversal potential, \(G_{\infty}\) is the maximum Ca\(^{2+}\) channel conductance, \(V_{1/2}\) is the half-maximal activation potential and \(k_c\) is the slope factor. The deactivation phase of SkEIIIK tail current was fitted as:

\[
I(t) = A[\exp(-t/\tau)] + C,
\]

(Eq. 2)

where \(I(t)\) is the current at time \(t\) after the repolarization, \(A\) is the peak tail current amplitude, \(\tau\) is the deactivation time constant, and \(C\) represents the steady current.\(^7\) All electrophysiological experiments were performed at room temperature (~25°C).

**Ca\(^{2+}\) imaging.** In order to block SR Ca\(^{2+}\) release, all cells used in Ca\(^{2+}\) imaging experiments were pretreated with 200 μM ryanodine for ~1 hr at 37°C. Myotubes were washed with Ca\(^{2+}\)/Mg\(^{2+}\)-free Ringer’s solution (in mM: 146 NaCl, 5 KCl, 10 HEPES, 11 glucose, pH 7.4 with NaOH) twice and subsequently loaded with 5 μM Fluo-3-AM (Molecular Probes, Eugene, OR, #F-1242) dissolved in Rodent Ringer’s solution (in mM: 146 NaCl, 5 KCl,
2 CaCl₂, 1 MgCl₂, 10 HEPES, pH 7.4 with NaOH) for 20 minutes. Myotubes were then washed 3X in Rodent Ringer’s solution. Fluoro-3-AM-loaded myotubes bathed in Rodent Ringer’s solution (−25°C) were then placed on the stage of an LSM META scanning laser confocal microscope (Zeiss, Thornwood, NY) and viewed with 10X magnification. N-Benzyl-P-toluensulfonamide (BTS; 10–100 μM; #S949760, Sigma, St. Louis, MO) was continuously present in the bath. Fluoro-3 was excited with the 488-nm line of an argon laser (30-milliwatt maximum output, operated at 50% or 6.3 A, attenuated to 1–2%). The emitted fluorescence was directed to a photomultiplier equipped with a 505-nm long-pass filter. Confocal fluorescence intensity data were digitized at 8-bits, with the photomultiplier gain and offset adjusted such that maximum pixel intensities were no more than ~70% saturated and cell-free areas had close to zero intensity. Ca²⁺ transients were elicited by application of 60 mM K⁺ Ringer’s solution (in mM: 91 NaCl, 60 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, pH 7.4 with NaOH) via a manually-operated, gravity-driven global perfusion system which produced complete exchange within ~5 s. Fluorescence data are expressed as ΔF/F, where F represents the baseline fluorescence prior to application of high K⁺ Ringer’s solution and ΔF represents the change in peak fluorescence during the application of high K⁺ Ringer’s solution. \( t_{\text{peak/2}} \) was calculated as the time required for the transient to reach half-peak amplitude measured from the onset of the initial upswing of the transient.

Pharmacology. Ryanodine (#Asc-083, Ascent Biosciences, Princeton, NJ) was reconstituted at 20 mM in 40% EtOH and diluted to 200 μM in differentiation medium. Nifedipine (#481981, Calbiochem, La Jolla, CA) was dissolved in 50% EtOH at 10 mM and diluted to 10 or 50 μM in Rodent Ringer’s solution just prior to experiments. In some experiments, Ca²⁺ currents were recorded following application of racemic Bay K 8644 (kindly supplied by Dr. A. Scriabine, Miles Laboratories Inc., New Haven, CT). Racemic Bay K 8644 was stored as a 20 mM stock in 50% EtOH. Dihydropyridines were stored and used in the dark.

Analysis. Figures were made using the software program SigmaPlot (version 7.0, SSPS Inc., Chicago, IL). All data are presented as mean ± SEM. Statistical comparisons were by unpaired, two-tailed t-test, with p < 0.05 considered significant.

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