Ca^{2+} Current Activation Rate Correlates with α_1 Subunit Density

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ABSTRACT We report here that L-type Ca^{2+} channels activate rapidly in myotubes expressing current at high density and slowly in myotubes expressing current at low density. Partial block of the current in individual cells does not slow activation, indicating that Ca^{2+} influx does not link activation rate to current density. Activation rate is positively correlated with the density of gating charge (Q_{max}) associated with the L-type Ca^{2+} channels. The range of values for Q_{max}, and the relationship between activation rate and Q_{max}, are similar for myotubes expressing native or recombinant L-type Ca^{2+} channels, whereas peak Ca^{2+} current density is ~3-fold higher for native channels. Taken together, these results suggest that Ca^{2+} channel density can govern activation kinetics. Our findings have important implications for studies of ion channel function because they suggest that biophysical properties can be significantly influenced by channel density, both in heterologous expression systems and in native tissues.

INTRODUCTION

The skeletal muscle dihydropyridine receptor is now known to be the α_1 subunit (α_1S) of the skeletal muscle L-type Ca^{2+} channel (Hoffmann et al., 1994). Previous experiments have established that α_1S functions as the voltage-sensing, drug-binding, and pore-forming Ca^{2+} channel subunit (Tanabe et al., 1988; Perez-Reyes et al., 1989) and as the "voltage sensor" for excitation-contraction (E-C) coupling in skeletal muscle (Rios and Brum, 1987; Tanabe et al., 1988). The macroscopic currents mediated by the skeletal muscle L-type Ca^{2+} channel activate with unusual slowness and are thus kinetically distinct from currents carried through other Ca^{2+} channel types (Sanchez and Stefani, 1978; Donaldson and Beam, 1983).

A number of different conditions have been shown to influence the activation kinetics of voltage-gated Ca^{2+} channels. These include the primary sequence of the α_1 subunit (Tanabe et al., 1991; Nakai et al., 1994), the previous voltage history of the channels (Hoshi et al., 1984; Artalejo et al., 1990; Feldmeyer et al., 1990; Pietrobon and Hess, 1990), the phosphorylation state of the channels (Sculptoreanu et al., 1993), interactions of the channels with G-protein-dependent pathways (Hille, 1994), and the presence or absence of ancillary Ca^{2+} channel subunits, especially the β subunit (for reviews, see Snutch and Reiner, 1992; Bean, 1994).

In our experiments with normal mouse myotubes and with dysgenic myotubes transfected with the rabbit α_1S subunit, we have observed a correlation between the activation rate of the macroscopic skeletal muscle L-type Ca^{2+} current and the density of the underlying Ca^{2+} channels. In the present paper we have investigated the nature of this relationship. We demonstrate here that the activation rate of macroscopic Ca^{2+} currents is independent of Ca^{2+} influx but is significantly correlated with the expression density of the α_1S subunits, as determined by measurements of maximum gating charge.

MATERIALS AND METHODS

Primary cultures of myotubes were prepared from newborn normal mice or mice with muscular dysgenesis, as previously described (Adams and Beam, 1989). Approximately 7 days after plating, the nuclei of developing dysgenic myotubes were microinjected with the expression plasmid pCAC6, which carries the cDNA encoding the rabbit α_1S subunit, also known as the skeletal muscle dihydropyridine receptor (Tanabe et al., 1987). One to three days later, while still bathed in the culture medium, injected myotubes were tested for the ability to contract in response to electrical stimulation via a saline/agar-filled extracellular pipette (Tanabe et al., 1988). The culture medium was then replaced by external solution (see below for composition), and ionic Ca^{2+} channel currents and intramembrane charge movements were recorded by the whole-cell patch-clamp technique (Hamill et al., 1981) as previously described (Adams et al., 1990). After establishment of the whole-cell configuration, electronic compensation was used to minimize the access resistance (usually to <1 MΩ) and the time required to charge the cell capacitance (usually to <0.5 ms). The steady holding potential was −80 mV. Test pulses were delivered at 5-s intervals, and each test pulse was immediately preceded by a 1-s prepulse to −30 mV to inactivate endogenous T-type Ca^{2+} current (Beam et al., 1986). Linear membrane capacitance and leakage currents were measured for each myotube during ten voltage steps from −80 to −100 mV; these control currents were averaged, scaled appropriately, and subtracted from test currents. Ca^{2+} current densities (expressed in pA/pF) were calculated for each myotube by dividing the leak- and capacitance-corrected test currents by whole-cell linear capacitance. The time constant for activation (τ_{act}) of ionic Ca^{2+} current was derived by fitting a single exponential function to the activating segment of test currents recorded at

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or slightly positive to the peak of the current-voltage (*I-V*) relation (Tanabe et al., 1991).

Gating currents (intramembrane charge movements) were recorded after the addition of 0.5 mM Cd^{2+} and 0.1 mM La^{3+} to the external solution; this combination of Cd^{2+} and La^{3+} effectively blocked ionic Ca^{2+} currents carried through Ca^{2+} channels. To isolate gating currents arising from voltage-dependent transitions of α_1S, we employed a voltage protocol modified from the method of Bean and Rios (1989). With this protocol, membrane potential was stepped from the holding potential (−80 mV) to −30 mV for 1 s, then to −50 mV for 20–30 ms, and finally to a variable test potential. As previously demonstrated (Adams et al., 1990), this protocol inactivates endogenous voltage-gated Na^+ current and T-type Ca^{2+} current and immobilizes the components of gating current that presumably arise from Na^+ channels and T-type Ca^{2+} channels. The "immobilization-resistant" gating currents that moved in response to test pulses were corrected for linear leakage and capacitative currents by subtracting an averaged, appropriately scaled control current, obtained during ten voltage steps between −80 and −140 mV. The amplitude of these control voltage steps was adjusted for each cell to maximize the signal-to-noise ratio of the corrected gating currents. To prevent amplifier saturation, voltage clamp command pulses were exponentially rounded with a time constant of 50–300 μs. For a given test pulse, the amount of charge that moved outward after the onset of the test pulse (Q_{on}) was obtained by integration. Q_{max}, the maximum amount of charge that could be moved, was taken as Q_{on} for a test pulse to +30 or +40 mV. Maximum gating charge density (expressed in nC/μF) was calculated by dividing Q_{max} recorded from each myotube by that cell's linear capacitance.

Patch pipettes were filled with a solution containing (in mM) 140 Cs-aspartate, 10 Cs_2EGTA, 5 $MgCl_2$, and 10 HEPES, with pH adjusted to 7.4 with CsOH or HCl. When immersed in the external solution, filled pipettes had resistances of 1.5–2.1 MΩ. The external solution contained (in mM) 145 tetraethylammonium chloride, 10 $CaCl_2$, 0.003 tetrodotoxin, and 10 HEPES, with pH adjusted to 7.4 with tetraethylammonium hydroxide or HCl. All experiments were performed at room temperature (20–23°C).

The data presented in Figs. 2, 3, 6, and 7 were analyzed using linear regression, and the significance of the Pearson product-moment correlation coefficient *r* was determined by using a two-tailed *t*-test (Glantz, 1981).

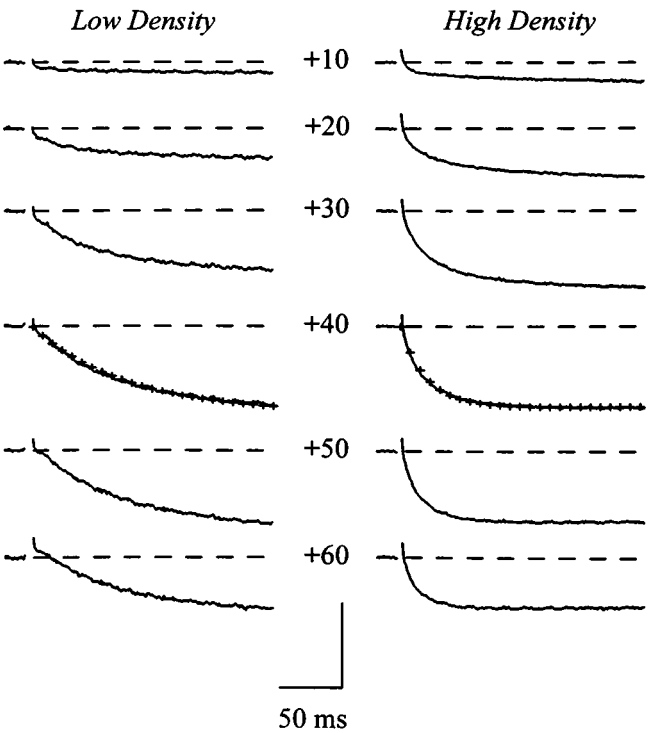


FIGURE 1 Activation is faster for higher-density Ca^{2+} currents. Whole-cell Ca^{2+} currents were recorded from two pCAC6-injected dysgenic myotubes. Test potentials are indicated next to current traces. (*Left*) Dysgenic myotube BP75, linear capacitance (*C*) = 370 pF. (*Right*) Dysgenic myotube BK31, *C* = 600 pF. Vertical calibration equals 1 nA (low density) and 4.17 nA (high density). Single exponential functions with time constants of 75 ms (low density) and 21 ms (high density) are shown superimposed on the peak currents (elicited by depolarizations to +40 mV).

RESULTS

Dysgenic myotubes that have been injected with an expression plasmid (pCAC6) encoding the rabbit α_1S subunit express L-type Ca^{2+} currents that are nearly identical to native L-type Ca^{2+} currents recorded from normal mouse myotubes (Tanabe et al., 1988). Although currents mediated by α_1S activate slowly in both normal and pCAC6-injected dysgenic myotubes, the time constant for activation (τ_{act}) of the current can vary over nearly a 10-fold range (Tanabe et al., 1991). In the present study, we have attempted to understand the basis for this variability in τ_{act}.

Fig. 1 shows currents recorded from two different dysgenic myotubes injected with pCAC6: the records on the left are from a myotube expressing a relatively low density of current (2.6 pA/pF), and those on the right are from one expressing a significantly higher density of current (6.6 pA/pF). Activation was much slower in the cell expressing a low-density current. Because a single exponential function provided a reasonable description of activation time course, the time constant (τ_{act}) determined with such a fit was used to quantify differences between cells. As a way of minimizing contributions of cell-to-cell variations in the voltage dependence of activation, comparisons of τ_{act} used the value determined for each cell at the test potential eliciting the maximum current. This test potential was +40 mV for the two cells illustrated in Fig. 1, and a single exponential function (*crosses*) is shown superimposed on the +40 mV trace for each cell. Because τ_{act} is only weakly voltage dependent for test pulses greater than or equal to that eliciting peak current (Dirksen and Beam, 1995), the differences in τ_{act} between cells did not depend strongly upon the potential used for comparison. For example, for the currents illustrated in Fig. 1, at test potentials of +40, +50, +60, and +70 mV, respectively, τ_{act} had values of 75, 71, 66, and 65 ms (low-density cell) compared with values of 21, 15.1, 13.4, and 12.1 ms (high-density cell). This weak voltage dependence of τ_{act} means that the different activation rates of low-density and high-density currents cannot be easily explained by a difference in the voltage dependence of activation.

We observed a consistent, inverse relationship between the value of τ_{act} and the maximum density of L-type Ca^{2+} current in pCAC6-expressing dysgenic myotubes and in normal mouse myotubes expressing the native L-type Ca^{2+} channel (Fig. 2). Although current densities were lower in pCAC6-expressing dysgenic myotubes, values of τ_{act} were very similar to those of normal myotubes. Consequently, the slope of the regression between τ_{act} and current density was

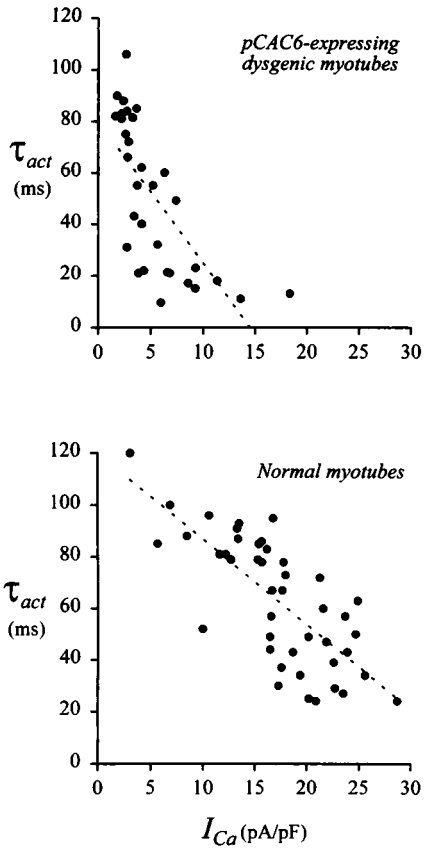


FIGURE 2 Time constants for activation (τ_{act}) depend on current density. τ_{act} was determined for the maximum L-type Ca^{2+} current recorded from each myotube and is shown plotted against the density of that current. For pCAC6-expressing dysgenic myotubes (n = 32), the relationship between τ_{act} and current density (ρ) is described by the equation $\tau_{act} = 80 - 5.5\rho$, with a Pearson product-moment correlation coefficient (r) of -0.70 ($p < 0.001$). For normal myotubes (n = 44), the relationship between τ_{act} and ρ is described by the equation $\tau_{act} = 120 - 3.3\rho$; $r = -0.75$ ($p < 0.001$).

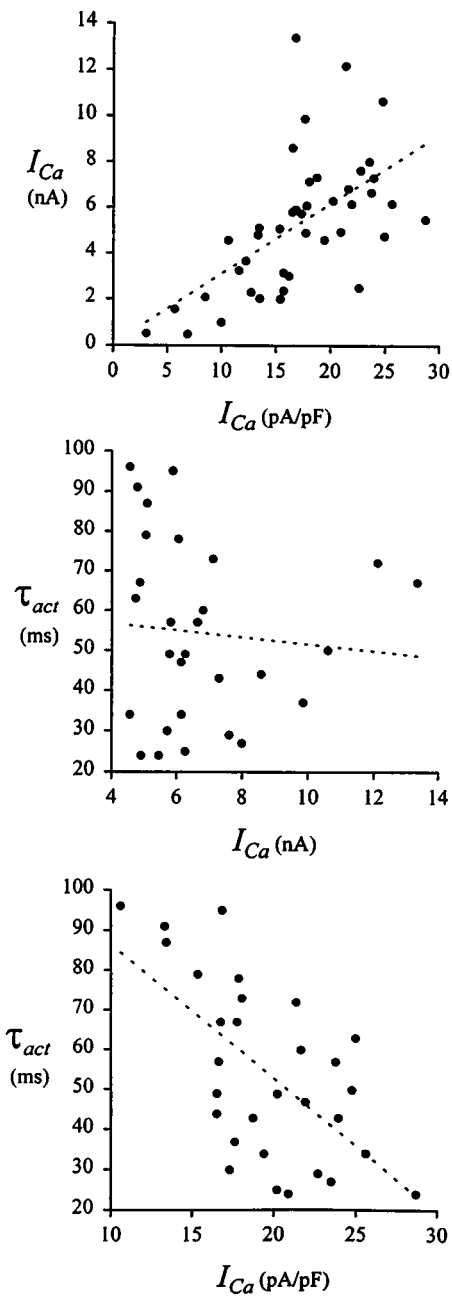


FIGURE 3 (*Top*) Absolute current amplitude and current density are correlated (n = 44 normal myotubes; $r = 0.59$, $p < 0.001$). (*Middle*) τ_{act} is not correlated with absolute current amplitude ($r = 0.083$, $p > 0.5$) in 30 normal myotubes expressing currents of 4 nA or more. (*Bottom*) τ_{act} and current density are significantly correlated ($r = 0.622$, $p < 0.001$) in these same 30 normal myotubes.

much steeper for pCAC6-expressing dysgenic myotubes (-5.5) than for normal myotubes (-3.3).

Not surprisingly, cells that express high-density Ca^{2+} currents also tend to express currents with large absolute amplitudes (Fig. 3, *top*). This correlation between current amplitude and density raises the possibility that a systematic, amplitude-dependent distortion of activation kinetics might account for the correlation between τ_{act} and current density (Fig. 2). To examine this possibility, we plotted τ_{act} as a function of current amplitude for 30 normal myotubes that expressed currents with an absolute amplitude of 4 nA or greater (Fig. 3, *middle*). Because distortion of activation kinetics should increase in proportion to the product of absolute current amplitude and series resistance, it is expected that any current-dependent distortion of kinetics would be most apparent in these cells. However, linear regression analysis showed that there was no correlation ($r = 0.0863$, $p > 0.5$) between τ_{act} and absolute current amplitude in these 30 normal myotubes. By way of contrast, there was a highly significant correlation ($r = 0.622$, $p <$ 0.001) between τ_{act} and current density in these same cells (Fig. 3, *bottom*). These results suggest that τ_{act} is correlated with current density rather than current amplitude, and furthermore, that the correlation between τ_{act} and current density cannot be explained by series resistance artifacts in the voltage clamp.

The relationship between τ_{act} and current density suggested that Ca^{2+} influx might somehow speed macroscopic

current activation. To test this hypothesis, we determined whether activation rate was slowed when the density of L-type current in individual myotubes was reduced by three separate experimental treatments: lowering the extracellular Ca^{2+} concentration ($n = 3$), partially blocking the current with extracellular Cd^{2+} ($n = 3$), or application of a long depolarizing prepulse to partially inactivate the underlying Ca^{2+} channels ($n = 3$). As illustrated in Fig. 4, each of these treatments substantially reduced the current density without appreciably altering activation kinetics. Because activation was not exceptionally fast in these myotubes, we also partially blocked the L-type current in a pCAC6-expressing dysgenic myotube (Fig. 5). Although activation was quite fast in this particular cell, reduction of the current failed to slow activation. From these results, we conclude that Ca^{2+} influx per se does not govern activation of the L-type current.

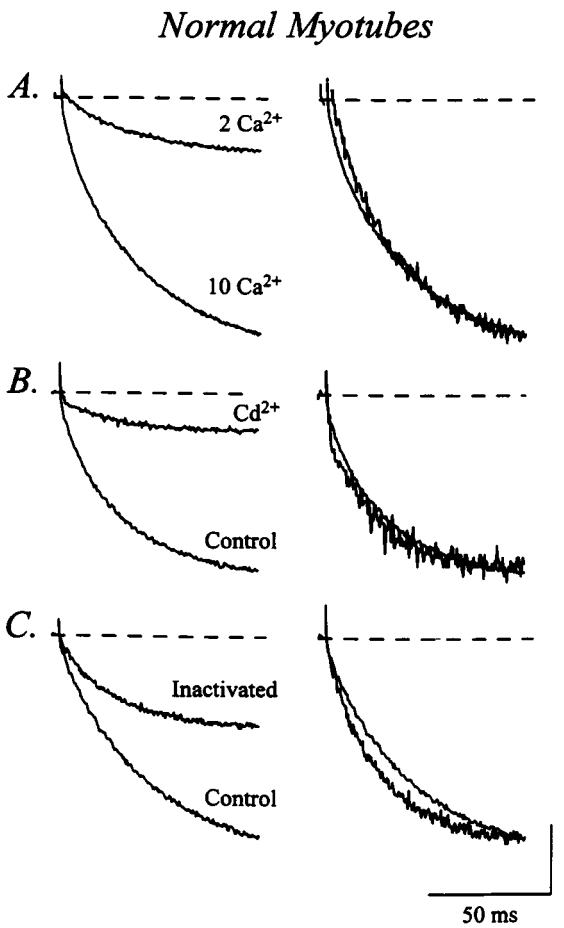


FIGURE 4 τ_{act} is unaffected by partial blockade of the skeletal muscle L-type Ca^{2+} current. Control and partially blocked currents are shown to scale on the left side of the figure. Partially blocked currents are shown scaled (noisy traces) and superimposed on the control currents on the right side of the figure. (*A*) External [Ca^{2+}] was decreased from 10 to 2 mM (8 mM Mg^{2+} was included in the 2 mM [Ca^{2+}] solution). τ_{act} was 61 ms for both control and reduced currents. Maximum current densities were 21.3 pA/pF in 10 mM Ca^{2+} and 4.7 pA/pF in 2 mM Ca^{2+}. Normal myotube BR88, C = 400 pF. (*B*) 25 μM $CdCl_2$ was added to the external solution. τ_{act} was 51 ms for the control current and 50 ms for the slow component of the partially blocked current. Control current density was 12.9 pA/pF. Normal myotube BR82, C = 200 pF. (*C*) The holding potential was changed from −80 to 0 mV for 60 s, then returned to −80 mV for 220 s preceding the test pulse. τ_{act} was 73 ms for the control and 51 ms for the partially inactivated current. Control current density was 14.6 pA/pF. Normal myotube BR83, C = 200 pF. Vertical calibration is 2.5 nA (*A*) or 1 nA (*B* and *C*). Test pulses were to +30 mV in all cases.

As noted above, the slope of the regression of τ_{act} versus current density is much steeper in pCAC6-expressing dysgenic myotubes than in normal myotubes (Fig. 2). One possible explanation for this difference is that the process-(es) underlying the correlation between current density and activation rate may differ in these two cell types. Another possibility is that some parameter other than current density is the most important determinant of activation. Current density is described by the expression NP_oi, where N is the density of channel proteins, P_o is the open probability, and i is the single-channel current. If the amount of gating charge moved is similar for channels with different P_o's, then the density of channel proteins (N) can be estimated from Q_{max}, the maximum gating charge (Adams et al., 1990; Neely et al., 1993). On this basis, the density of L-type channel proteins is similar in normal and pCAC6-injected myotubes, because Q_{max} averaged 6.8 and 6.0 nC/μF in these cell types, respectively (see also Adams et al., 1990). However, as shown in Fig. 6, the relationship between Ca^{2+} current density and Q_{max} was dramatically shifted to lower current densities in pCAC6-expressing dysgenic myotubes compared to normal myotubes, suggesting that maximum P_o is considerably lower in injected dysgenic myotubes.

Although there is considerable scatter in the data, linear regression indicates that the relationship between τ_{act} and Q_{max} has a similar slope in normal and pCAC6-injected myotubes (Fig. 7). This result is consistent with the idea that channel density, rather than current density, is the important determinant of activation rate. If this idea is correct, then the difference in regression slope for τ_{act} versus current density observed between normal and pCAC6-injected myotubes

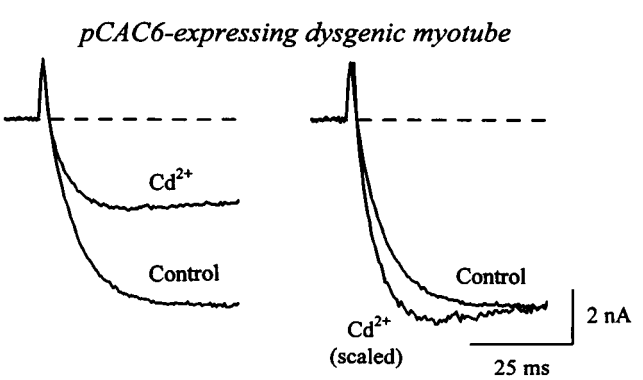
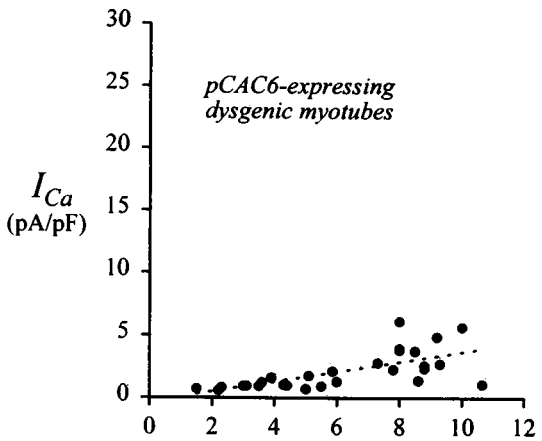
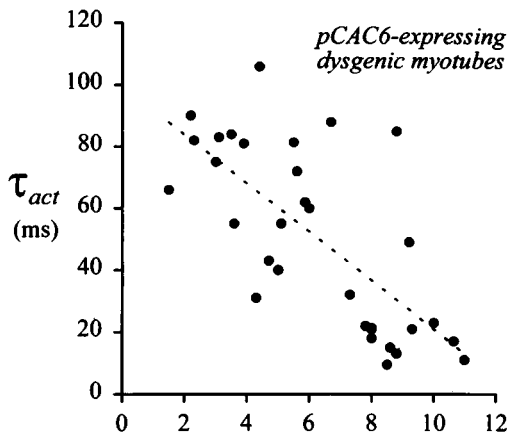
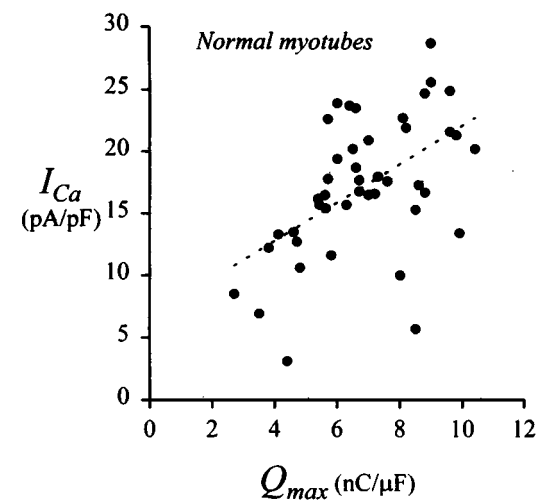


FIGURE 5 Partial block of a relatively fast-activating current does not slow activation. Skeletal muscle L-type current recorded from a pCAC6-expressing dysgenic myotube was experimentally reduced by the addition of 25 μM $CdCl_2$ to the external solution. Dysgenic myotube BW38, C = 940 pF. The control current had a density of 6.6 pA/pF and a τ_{act} of 12 ms; τ_{act} was 8 ms for the Cd^{2+}-blocked current.







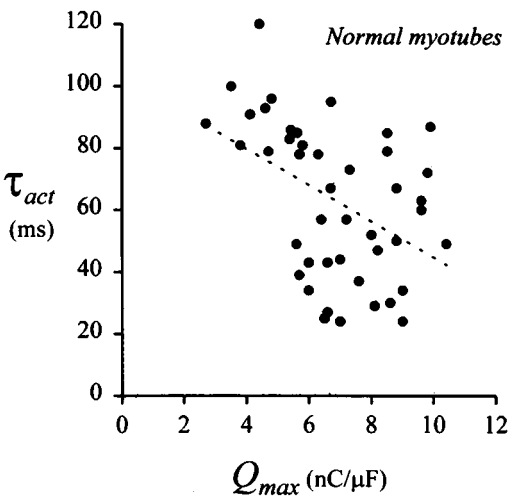


FIGURE 6 Ca^{2+} current density is linearly related to the gating charge density. Maximum L-type Ca^{2+} current density is shown plotted as a function of maximum charge movement density Q_{max} recorded from the same myotube. The linear regression for pCAC6-expressing dysgenic myotubes (n = 32) was $I_{Ca} = -0.28 + 0.4(Q_{max})$; $r = 0.68$; $p < 0.001$. For normal myotubes (n = 44), the linear regression was $I_{Ca} = 6.6 + 1.55(Q_{max})$; $r = 0.53$; $p < 0.001$.

FIGURE 7 τ_{act} is inversely related to Q_{max}. The linear regression for pCAC6-expressing dysgenic myotubes (n = 32) was $\tau_{act} = 100 - 7.9(Q_{max})$; $r = 0.71$, $p < 0.001$. The linear regression for normal myotubes (n = 44) was $\tau_{act} = 103 - 5.9(Q_{max})$; $r = 0.45$, $p < 0.005$.

(Fig. 2) is a consequence of the difference in maximum P_o for L-type channels expressed in the two cell types.

DISCUSSION

In this paper we have shown that in both normal and transfected myotubes, activation kinetics of the skeletal muscle L-type Ca^{2+} current are more rapid in cells expressing a higher density of channels. This result does not appear to be a consequence of Ca^{2+} influx or an electrophysiological artifact, because activation kinetics were little affected by manipulations that reduced current amplitude. To the best of our knowledge, this is the first demonstration of a link between the expression density of Ca^{2+} channel proteins and Ca^{2+} channel activation kinetics. These results are especially significant, considering the wide number of studies that use heterologous systems to investigate various properties of ion channels expressed from their cDNAs.

Previously we demonstrated that the activation rate of the skeletal muscle L-type Ca^{2+} channel is determined by the amino acid sequence of a specific region (IS3 and the IS3-IS4 linker) within the first transmembrane domain of the α_1S subunit (Nakai et al., 1994). Our present results demonstrating a relationship between τ_{act} and channel density complement, rather than contradict, these previous structural findings. For any given chimera of the cardiac and skeletal muscle α_1 subunits, we observed variation in both current density and activation kinetics, yet each chimera could be clearly classified as displaying either distinctly fast or distinctly slow activation (Tanabe et al., 1991; Nakai et al., 1994). Thus, the effect of channel density on activation rate appears to be superimposed upon the more important effect of channel structure.

Several previous studies have demonstrated a connection between channel density and activation kinetics. For exam-

ple, Delbono (1992) reported that L-type Ca^{2+} current density, activation rate, and intramembrane charge movement density were all decreased in denervated rat skeletal muscle fibers. Paradoxically, average current density was slightly increased, but activation rate was decreased 4–6 days after denervation, whereas both activation rate and current density were decreased at later times after denervation. The apparent uncoupling of activation rate and current density at short times after denervation might be explained by variations in current density among different groups of muscle fibers, because it is unclear in Delbono (1992) whether activation, current density, and charge movement were all measured in the same group of cells, as was done in the present study (Figs. 2, 3, 6, and 7). In another example, Very et al. (1994) reported that current amplitudes were larger and activation was faster when more *Arabidopsis* KAT1 inward rectifier K^+ channels were expressed in *Xenopus* oocytes. A relationship between channel expression level and activation kinetics has also been described for MinK potassium channels expressed in *Xenopus* oocytes (Blumenthal and Kaczmarek, 1994; Cui et al., 1994). However, in the case of MinK channels, activation was slower when more channels were expressed. Thus, the mechanism linking MinK channel density to activation rate is probably not the same as that for L-type Ca^{2+} channels or KAT1 channels. These studies each suggest, despite their differences, that the channel expression level can have significant effects on channel gating kinetics.

It is possible that increased channel density in and of itself causes activation to be faster. For example, channel activation might be cooperative, either as a consequence of direct interactions between the channels themselves or as a consequence of interactions with other proteins, such as the ryanodine receptor. However, our previous results with chimeras of α_1S and α_1C suggest that interactions with the ryanodine receptor do not influence activation rate, because values of τ_{act} were indistinguishable between chimeras that interacted with ryanodine receptors (i.e., mediated skeletal muscle-type E-C coupling) and those that did not (Tanabe et al., 1990). Furthermore, if there were cooperative interactions between adjacent channels that affected voltage-dependent transitions involved in channel activation, one would expect that strong depolarization would cause activation rates to be similar in cells of varying channel density, which does not seem to be the case (Fig. 1). In addition, channel opening per se does not seem to play a role in establishing activation rate, because τ_{act} is similar in normal and pCAC6-injected myotubes at a given channel density (i.e., Q_{max}; Fig. 7), despite a much higher maximum P_o in normal myotubes (as reflected in the slope of peak current density versus Q_{max}; Fig. 6).

Perhaps activation rate and channel density are not causally related but are instead both governed by a common factor. For example, association of the α_1S subunit with one or more of the ancillary Ca^{2+} channel subunits (α_2, β, or γ) might facilitate the insertion of the channel into the surface membrane and might result in a channel with faster activation. A number of recent studies have reported that coexpression of the Ca^{2+} channel β subunit with the α_1 subunit increases the amplitude of the expressed Ca^{2+} channel current and changes activation rate (Lacerda et al., 1991; Varadi et al., 1991; Singer et al., 1991; Wei et al., 1991; Hullin et al., 1992; Perez-Reyes et al., 1992; Perez-Garcia et al., 1995). Furthermore, several studies have demonstrated that coexpression of the α_2 subunit with α_1 and β greatly enhances channel expression (Mori et al., 1991; Hullin et al., 1992; Brust et al., 1993; Gurnett et al., 1996). These observations suggest that cell-to-cell variation in the level of ancillary subunit expression, or in the degree of association between α_1S and the ancillary subunits, could account for a correlation between channel density and activation rate. However, if the total current represents an algebraic sum of currents contributed by α_1S subunits associated with ancillary subunits and α_1S subunits not associated with ancillary subunits, then the total current ought to display both rapidly and slowly activating components. In some cells and at some test potentials, the skeletal muscle L-type current did seem to display two components of activation, but in a great many instances the activation of currents could be well described by a single exponential function.

Our findings do not allow us to determine whether τ_{act} varies as a continuous function of channel density or whether two separate populations of channels exist (slowly activating and rapidly activating), with the relative proportion of each type varying as a function of overall channel density. In principal, this question might be addressed by testing whether the currents could be better fit by varying the proportion of a fixed slow and a fixed fast exponential. However, in practice this test is unreliable because values of τ_{act} span a relatively small range (~5-fold), and the Ca^{2+} current displays biphasic activation kinetics at weak test potentials. Such an analysis is further confounded by the presence in dysgenic myotubes (and possibly normal myotubes as well) of an endogenous, rapidly activating Ca^{2+} current (I_{dys}), which is clearly distinct from the slowly activating current mediated by α_1S. As an alternative to the idea that association with ancillary subunits or interchannel interaction is the common factor affecting both channel density and activation rate, one might suppose that other cellular conditions could affect both variables. Among the many possibilities are phospholipid composition of the sarcolemma, association of the L-type channel with cytoskeletal proteins or G-proteins, and phosphorylation or other posttranslational modifications of the channel.

Heterologous expression of ion channels often results in wide variations in channel density among different transfected cells. We have shown here that the kinetic behavior of skeletal muscle L-type Ca^{2+} channels varies with the expression level of the α_1S subunits. These results have important implications for studies of ion channel function because they suggest that biophysical properties can be significantly influenced by channel density, both in heterologous expression systems and in native tissues. Thus, it may be worthwhile for future studies to consider the poten-

tial effect of variations in expression level on the biophysical properties of expressed channels.

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REFERENCES

Adams, B. A., and K. G. Beam. 1989. A novel Ca^{2+} current in dysgenic skeletal muscle. *J. Gen. Physiol.* 94:429–444.

Adams, B. A., T. Tanabe, A. Mikami, S. Numa, and K. G. Beam. 1990. Intramembrane charge movement restored in dysgenic skeletal muscle by injection of dihydropyridine receptor cDNAs. *Nature.* 346:569–572.

Artalejo, C. R., M. A. Ariano, R. L. Perlman, and A. P. Fox. 1990. Activation of facilitation Ca^{2+} channels in chromaffin cells by D1 dopamine receptors through a cAMP/protein kinase A-dependent mechanism. *Nature.* 348:239–242.

Beam, K. G., C. M. Knudson, and J. A. Powell. 1986. A lethal mutation in mice eliminates the slow Ca^{2+} current in skeletal muscle cells. *Nature.* 320:168–170.

Bean, B. P. 1994. Taking the beta test. *Nature.* 368:15–16.

Bean, B. P., and E. Rios. 1989. Nonlinear charge movement in mammalian cardiac ventricular cells. Components from Na and Ca channel gating. *J. Gen. Physiol.* 94:65–93.

Blumenthal, E. M., and L. K. Kaczmarek. 1994. The MinK potassium channel exists in functional and nonfunctional forms when expressed in the plasma membrane of *Xenopus* oocytes. *J. Neurosci.* 14:3097–3105.

Brust, P. F., S. Simerson, A. F. McCue, C. R. Deal, S. Schoonmaker, M. E. Williams, G. Velicelebi, E. C. Johnson, M. M. Harpold, and S. B. Ellis. 1993. Human neuronal voltage-dependent calcium channels: studies on subunit structure and role in channel assembly. *Neuropharmacology.* 32:1089–1102.

Cui, J., R. P. Kline, P. Pennefather, and I. S. Cohen. 1994. Gating of *Isk* expressed in *Xenopus* oocytes depends on the amount of mRNA injected. *J. Physiol.* 104:87–105.

Delbono, O. 1992. Calcium current activation and charge movement in denervated mammalian skeletal muscle fibers. *J. Physiol. (Lond.).* 451: 187–203.

Dirksen, R. T., and K. G. Beam. 1995. Single calcium channel behavior in native skeletal muscle. *Gen. Physiol.* 105:227–247.

Donaldson, P. L., and K. G. Beam. 1983. Calcium currents in fast-twitch skeletal muscle of the rat. *J. Gen. Physiol.* 82:449–468.

Feldmeyer, D., W. Melzer, B. Pohl, and P. Zollner. 1990. Fast gating kinetics of the slow Ca^{2+} current in cut skeletal muscle fibres of the frog. *J. Physiol. (Lond.).* 425:347–367.

Glantz, S. A. 1981. Primer of Biostatistics. McGraw-Hill, New York.

Gurnett, C. A., M. De Waard, and K. P. Campbell. 1996. Dual function of the voltage-dependent Ca^{2+} channel $\alpha 2\delta$ subunit in current stimulation and subunit interaction. *Neuron.* 16:431–440.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 391:85–100.

Hille, B. 1994. Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci.* 17:531–536.

Hoffmann, F., M. Biel, and V. Flockerzi. 1994. Molecular basis for Ca^{2+} channel diversity. *Annu. Rev. Neurosci.* 17:99–418.

Hoshi, T., J. Rothlein, and S. J. Smith. 1984. Facilitation of Ca^{2+}-channel currents in bovine adrenal chromaffin cells. *Proc. Natl. Acad. Sci. USA.* 81:5871–5875.

Hullin, R., D. Singer-Lahat, M. Freichel, M. Biel, N. Dascal, F. Hofmann, and V. Flockerzi. 1992. Calcium channel β-subunit heterogeneity: functional expression of cloned cDNA from heart, aorta and brain. *EMBO J.* 11:885–890.

Lacerda, A. E., H. S. Kim, P. Ruth, E. Perez-Reyes, V. Flockerzi, F. Hofmann, L. Birnbaumer, and A. M. Brown. 1991. Normalization of current kinetics by interaction between the α_1 and β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel. *Nature.* 352: 527–530.

Mori, Y., T. Friedrich, M.-S. Kim, A. Mikami, J. Nakai, P. Ruth, E. Bosse, F. Hofmann, V. Flockerzi, T. Furuichi, K. Mikoshiba, K. Imoto, T. Tanabe, and S. Numa. 1991. Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature.* 350: 398–402.

Nakai, J., B. A. Adams, K. Imoto, and K. G. Beam. 1994. Critical roles of the S3 segment and S3–S4 linker of repeat I in the activation of L-type Ca^{2+} channels. *Proc. Natl. Acad. Sci. USA.* 91:1014–1018.

Neely, A., X. Wei, R. Olcese, L. Birnbaumer, and E. Stefani. 1993. Potentiation by the β subunit of the ratio of the ionic current to the charge movement in the cardiac Ca^{2+} channel. *Science.* 262:575–578.

Perez-Garcia, M. T., T. J. Kamp, and E. Marban. 1995. Functional properties of cardiac L-type calcium channels transiently expressed in HEK293 cells. *J. Gen. Physiol.* 105:289–306.

Perez-Reyes, E., A. Castellano, H. S. Kim, P. Bertrand, E. Baggstrom, A. E. Lacerda, X. Wei, and L. Birnbaumer. 1992. Cloning and expression of a cardiac/brain beta subunit of the L-type calcium channel. *J. Biol. Chem.* 267:1792–1797.

Perez-Reyes, E., H. S. Kim, A. E. Lacerda, W. Horne, X. Wei, D. Rampe, K. P. Campbell, A. M. Brown, and L. Birnbaumer. 1989. Induction of Ca^{2+} currents by the expression of the α_1-subunit of the dihydropyridine receptor from skeletal muscle. *Nature.* 340:233–236.

Pietrobon, D., and P. Hess. 1990. Novel mechanism of voltage-dependent gating in L-type calcium channels. *Nature.* 346:651–655.

Rios, E., and G. Brum. 1987. Involvement of dihydropyridine receptors in excitation-contraction coupling. *Nature.* 325:717–720.

Sanchez, J. A., and E. Stefani. 1978. Inward calcium current in twitch muscle fibers of the frog. *J. Physiol. (Lond.).* 283:197–209.

Sculptoreanu, A., T. Scheuer, and W. A. Catterall. 1993. Voltage-dependent potentiation of L-type Ca^{2+} channels due to phosphorylation by cAMP-dependent protein kinase. *Nature.* 364:240–243.

Singer, D., M. Biel, I. Lotan, V. Flockerzi, F. Hofmann, and N. Dascal. 1991. The roles of the subunits in the function of the calcium channel. *Science.* 253:1552–1557.

Snutch, T. P., and P. B. Reiner. 1992. Ca^{2+} channels: diversity of form and function. *Curr. Opin. Neurobiol.* 2:247–253.

Swandulla, D., and C. M. Armstrong. 1989. Calcium channel block by cadmium in chick sensory neurons. *Proc. Natl. Acad. Sci. USA.* 86: 1736–1740.

Tanabe, T., B. A. Adams, S. Numa, and K. G. Beam. 1991. Repeat I of the dihydropyridine receptor is critical in determining Ca^{2+} channel activation kinetics. *Nature.* 352:800–803.

Tanabe, T., K. G. Beam, B. A. Adams, T. Niidome, and S. Numa. 1990. Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature.* 346:567–569.

Tanabe, T., K. G. Beam, J. A. Powell, and S. Numa. 1988. Restoration of excitation-contraction coupling and slow Ca^{2+} current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature.* 336: 134–139.

Tanabe, T., H. Takeshima, A. Mikami, V. Flockerzi, H. Takahashi, K. Kangawa, M. Kojima, H. Matsuo, T. Hirose, and S. Numa. 1987. Primary structure of the receptor for Ca^{2+} channel blockers from skeletal muscle. *Nature.* 328:313–318.

Varadi, G., P. Lory, D. Schultz, M. Varadi, and A. Schwartz. 1991. Acceleration of activation and inactivation by the β subunit of the skeletal muscle calcium channel. *Nature.* 352:159–162.

Very, A.-A., C. Bosseux, F. Gaymard, H. Sentenac, and J.-B. Thibaud. 1994. Level of expression in *Xenopus* oocytes affects some characteristics of a plant inward-rectifying voltage-gated K^+ channel. *Pflugers Arch.* 428:422–424.

Wei, X., E. Perez-Reyes, A. E. Lacerda, G. Shuster, A. M. Brown, and L. Birnbaumer. 1991. Heterologous regulation of the cardiac Ca^{2+} channel α_1 subunit by skeletal muscle β and γ subunits. *J. Biol. Chem.* 266: 21943–21947.