Heterologous Expression of BI Ca^{2+} Channels in Dysgenic Skeletal Muscle

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ABSTRACT We have examined the ability of BI (class A) Ca^{2+} channels, cloned from rabbit brain, to mediate excitation-contraction (E-C) coupling in skeletal muscle. Expression plasmids carrying cDNA encoding BI channels were microinjected into the nuclei of dysgenic mouse myotubes grown in primary culture. Ionic currents and intramembrane charge movements produced by the BI channels were recorded using the whole-cell patch-clamp technique. Injected myotubes expressed high densities of ionic BI Ca^{2+} channel current (average 31 pA/pF) but did not display spontaneous contractions, and only very rarely displayed evoked contractions. The expressed ionic current was pharmacologically distinguished from the endogenous L-type current of dysgenic skeletal muscle (I_{dys}) by its insensitivity to the dihydropyridine antagonist (+)-PN 200–110. Peak BI Ca^{2+} currents activated with a time constant (τ_a) of ~2 ms and inactivated with a time constant (τ_h) of ~260 ms (20–23°C). The time constant of inactivation (τ_h) was not increased by substituting Ba^{2+} for Ca^{2+} as charge carrier, demonstrating that BI channels expressed in dysgenic myotubes do not undergo Ca^{2+}-dependent inactivation. The average maximal Ca^{2+} conductance (G_{max}) produced by the BI channels was quite large (~534 S/F). In contrast, the average maximal charge movement (Q_{max}) produced in the same myotubes (~2.7 nC/μF) was quite small, being barely larger than Q_{max} in control dysgenic myotubes (~2.3 nC/μF). Thus, the ratio G_{max}/Q_{max} for the BI channels was considerably higher than previously found for cardiac or skeletal muscle L-type Ca^{2+} channels expressed in the same system, indicating that neuronal BI Ca^{2+} channels exhibit a much higher open probability than these L-type Ca^{2+} channels.

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INTRODUCTION

The rabbit brain BI (class A) Ca^{2+} channel was the first neuronal Ca^{2+} channel to be expressed functionally from its complementary DNA (Mori, Friedrich, Kim, Mikami, Nakai, Ruth, Bosse, Hofmann, Flockerzi, Furuichi, Mikoshiba, Imoto, Tanabe, and Numa, 1991). When heterologously expressed in *Xenopus* oocytes, BI channels mediate an ionic current that is insensitive to dihydropyridine (DHP) antagonists and to ω-conotoxin GVIA, but that is partially blocked by crude venom from the funnel-web spider *Agelenopsis aperta* (Mori et al., 1991). More recently, BI channels expressed in oocytes have been shown to be partially suppressed by ω-Aga-IVA, a peptide component of *Agelenopsis* venom, and completely blocked by ω-conotoxin MVIIC (Sather, Tanabe, Zhang, Mori, Adams, and Tsien, 1993), a peptide predicted by cDNA cloned from the venom gland of *Conus magnus* (Hillyard, Monje, Mintz, Bean, Nadasdi, Ramachandran, Miljanich, Azimi-Zoonooz, McIntosh, Cruz, Imperial, and Olivera, 1992). Thus, the pharmacological sensitivities of expressed BI channels somewhat resemble those of native P-type Ca^{2+} channels found in mammalian Purkinje cell neurons (Llinas, Sugimori, Lin, and Cherksey, 1989; Regan, Sah, and Bean, 1991; Mintz, Venema, Swiderek, Lee, Bean, and Adams, 1992). However, quantitative differences in toxin sensitivity and biophysical properties between cloned BI channels and native P-type Ca^{2+} channels suggest that these two Ca^{2+} channels are not identical (Tsien, Ellinor, and Horne, 1991; Sather et al., 1993; Zhang, Randall, Ellinor, Horne, Sather, Tanabe, Schwarz, and Tsien, 1993; Wheeler, Randall, and Tsien, 1994).

The objectives of the present work were to examine the ability of BI Ca^{2+} channels to mediate excitation-contraction (E-C) coupling and to generate the intramembrane charge movement signal that is associated with this process in skeletal muscle (Rios and Pizarro, 1991). We also wanted to measure the ratio of Ca^{2+} conductance to intramembrane charge movement produced by gating of the BI channels, so that we could estimate the open probability (P_0) of the BI channels for comparison with L-type Ca^{2+} channels expressed in the same system. Previously, we have used myotubes prepared from skeletal muscle of mice with muscular dysgenesis as a system for investigating the mechanisms of E-C coupling and for characterizing the biophysical properties of cloned Ca^{2+} channels (Tanabe, Mikami, Niidome, Numa, Adams, and Beam, 1993). Dysgenic skeletal muscle is extremely useful for such experiments because it lacks excitation-contraction (E-C) coupling (Powell and Fambrough, 1973) and slowly-activating L-type Ca^{2+} current (Beam, Knudson, and Powell, 1986). Furthermore, dysgenic skeletal muscle lacks the high-density intramembrane charge movement that is characteristic of normal skeletal muscle (Adams, Tanabe, Mikami, Numa and Beam, 1990). However, when cDNAs encoding cloned L-type Ca^{2+} channels are expressed in dysgenic myotubes, E-C coupling, L-type Ca^{2+} currents and intramembrane charge movements are all restored, mirroring the physiological situations in cardiac and skeletal muscle (Tanabe et al., 1993).

L-type Ca^{2+} channels appear to exhibit a very low maximum probability of being open (P_0) during a depolarizing test pulse. This low P_0 has been revealed by single-channel measurements of native skeletal muscle Ca^{2+} channels reconstituted into lipid bilayers (Ma, Mundina-Weilenmann, Hosey, and Rios, 1991; Mejia-Alvarez,

Fill, and Stefani, 1991) and by cell-attached patch clamp recordings from skeletal muscle myotubes (Dirksen, Tanabe, and Beam, 1993) and cardiac ventricular cells (Lew, Hryshko, and Bers, 1991; Ono and Fozzard, 1992). A low P_0 is also reflected in the small ratio of maximum L-type Ca^{2+} conductance to intramembrane charge movement (G_{max}/Q_{max}) found in dysgenic myotubes exogenously expressing the cardiac or skeletal muscle L-type Ca^{2+} channels (Adams et al., 1990). We find that BI channels expressed in dysgenic myotubes produce a high-density Ca^{2+} conductance but a low density of intramembrane charge movement, suggesting that few BI channels are present in the membrane but that those present exhibit a relatively high P_0. Significantly, we also find that BI Ca^{2+} channels do not restore E-C coupling to dysgenic skeletal muscle. A preliminary report of these results has appeared (Adams, Tanabe, Mori, and Beam, 1994).

MATERIALS AND METHODS

Primary cultures of myotubes were prepared from newborn dysgenic mice as previously described (Adams and Beam, 1989). Approximately 7 d after plating, the nuclei of developing myotubes were microinjected (Tanabe, Beam, Powell, and Numa, 1988) with the expression plasmid pKCRCBI-1 or pKCRCBI-2, which carry cDNA encoding the BI-1 or BI-2 isoforms of the BI Ca^{2+} channel, respectively (Mori et al., 1991). The expression plasmid pKCRCBI-1, carrying the entire protein-coding sequence of the BI-1 Ca^{2+} channel, was constructed by inserting the *Hin*dIII(vector)/*Hin*dIII(7,932) fragment from pSPCBI-1 (Mori et al., 1991) into the *Hin*dIII site of pKCRH2 (Mishina, Kurosaki, Tobimatsu, Morimoto, Noda, Yamamoto, Terao, Lindstrom, Takahashi, Kuno, and Numa, 1984). The expression plasmid pKCRCBI-2, carrying the entire protein-coding sequence of the BI-2 Ca^{2+} channel, was constructed by inserting the *Hin*dIII(vector)/*Hin*dIII(7,519) fragment from pSPCBI-2 (Mori et al., 1991) into the *Hin*dIII site of pKCRH2. 1–3 d after injection with cDNA, myotubes bathed in culture medium 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 using the whole-cell patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) as previously described (Adams and Beam, 1989; Adams et al., 1990). After establishment of the whole-cell configuration, electronic compensation was used to minimize the effective series resistance (usually to <1 MΩ) and the time required to charge the cell capacitance (usually to <0.5 ms). The steady holding potential (HP) 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 10 voltage steps from -80 to -100 mV; these control currents (which directly preceded test 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 constants for activation (τ_a) and inactivation (τ_h) of expressed Ca^{2+} currents were derived by fitting single exponential functions to activating or inactivating segments, respectively, of test currents recorded near the peak of the current-voltage (I-V) relation. Maximal Ca^{2+} conductance (G_{max}) was calculated for individual myotubes by fitting measured values of inward current according to the expression

$$I = G_{max}(V - V_{rev})/\{1 + \exp[-(V - V_G)/k_G]\}$$

where I is the peak BI current activated at test potential V, V_{rev} is the extrapolated reversal potential, V_G is the potential for activation of half-maximal conductance, and k_G is a slope factor.

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. To isolate intramembrane charge movements (gating currents) arising from the expressed BI Ca^{2+} channels, we employed a voltage protocol modified from Bean and Rios (1989). With this protocol, membrane potential was stepped from the holding potential (−80 mV) to −30 mV for 1 s (the prepulse), then to −50 mV for 20–30 ms (the pedestal), and finally to a variable test potential. As previously demonstrated (Adams et al., 1990), this voltage protocol inactivates endogenous Na^+ current and T-type Ca^{2+} current and immobilizes a component of intramembrane charge movement, presumably that arising from these channels. Importantly, however, this voltage protocol had no effect on ionic Ca^{2+} currents produced by expression of the BI channels (see Fig. 4 *B*). The "immobilization-resistant" intramembrane charge movements recorded with this voltage protocol were corrected for linear leakage and capacitative currents by subtracting an averaged, appropriately scaled control current, obtained during 10 voltage steps between −80 and −140 mV. 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 charge movement density (expressed in nC/μF) was calculated by dividing Q_{max} for each cell by that cell's linear capacitance.

Patch pipettes were filled with a solution containing (in millimolar) 140 Cs-Aspartate, 10 HEPES, 10 Cs_2EGTA, 5 $MgCl_2$; pH 7.4 with CsOH. When immersed in the external solution, filled pipettes had resistances of 1.5–2.1 MΩ. The external solution contained (in millimolar) 145 tetraethylammonium (TEA) chloride, 10 HEPES, 10 $CaCl_2$ (or 10 $BaCl_2$ where indicated), 0.003 tetrodotoxin; pH 7.4 with TEA-OH. External solution containing 100 μM $CdCl_2$ was applied via a puffer pipette positioned within 500 μm of the cell. (+)-PN 200–110 (a gift from Drs. A. Lindenmann and E. Rossi of Sandoz Ltd., Basel, Switzerland) was applied by bulk exchange of the external solution. Before examination of the effects of (+)-PN 200–110, control currents were recorded after flushing the dish (~1 ml vol) with 10–15 ml of drug-free solution to control for perfusion effects on Ca^{2+} currents. Test currents were subsequently recorded after flushing the dish with 20–30 ml of solution containing 1 or 10 μM (+)-PN 200–110. (+)-PN 200–110 was prepared as a stock (10 mM in 100% ethanol) which was stored in the dark at −20°C; aliquots of this stock were diluted into the external solution immediately before use.

All experiments were performed at room temperature (20–23°C). Numerical values presented in the text are mean ± SEM with the number of observations in parentheses.

RESULTS

Dysgenic myotubes injected with pKCRCBI-1 or pKCRCBI-2 failed to display spontaneous or evoked contractions (see below). However, when we methodically patch clamped every myotube within the injected regions of the culture dishes, we found that ~10% expressed a high-density Ca^{2+} current. Fig. 1 illustrates a representative example of this current, which presumably was mediated by the expressed BI Ca^{2+} channels. The current activated rapidly with a time constant (τ_a) of 2.3 ± 0.1 ms (n = 38). The time course of inactivation (measured during a 1-s depolarization) was much slower, having a time constant (τ_h) of 258 ± 32 ms (n = 6).

With 10 mM Ca^{2+} as the charge carrier, the expressed BI Ca^{2+} current first appeared in response to test pulses to near −10 mV and peaked in response to test pulses to near +20 mV (Fig. 1 *B*). Because the properties of the expressed macroscopic currents were not different between myotubes injected with pKCRCBI-1 or pKRCBI-2, results obtained with these two expression plasmids have been combined.

The expressed current was mediated by Ca^{2+} channels, as demonstrated by its rapid and reversible block by 100 μM Cd^{2+} (Fig. 2 *A*). Furthermore, the BI Ca^{2+}

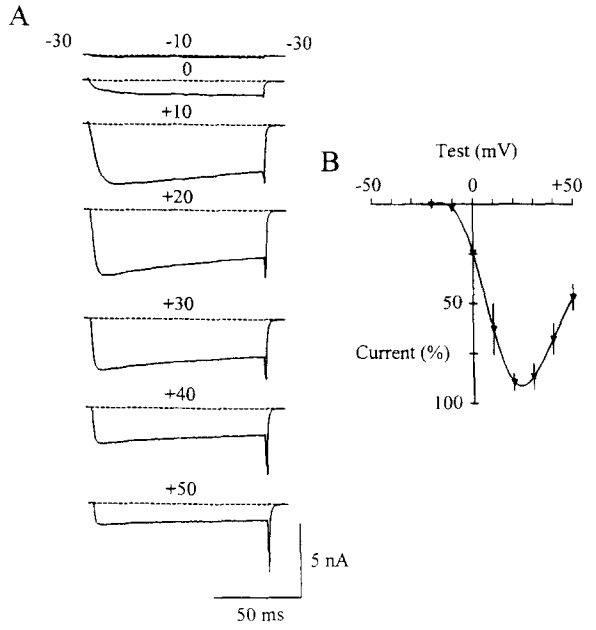

FIGURE 1. Kinetics and voltage dependence of BI Ca^{2+} currents expressed in dysgenic myotubes. (*A*) Representative BI currents. Test pulses to various potentials were immediately preceded by a 1-s prepulse to −30 mV in order to inactivate endogenous T-type Ca^{2+} current. Steady holding potential (HP) was −80 mV, and the charge carrier was 10 mM Ca^{2+}. Pretest, test, and repolarization potentials are all indicated above the current traces. The peak density of BI current recorded from this myotube was 28 pA/pF (measured at a test potential of +20 mV). The maximum calculated voltage error (V_{err}; calculated as the product of peak inward current and compensated series resistance) was 4.9 mV. Dysgenic myotube BT93, injected with pKCRCBI-2; linear capacitance (C) = 160 pF. (*B*) Average (±SEM) current-voltage (I-V) relationship of BI Ca^{2+} currents expressed in six dysgenic myotubes. Current amplitudes in each cell were normalized by the maximum current amplitude recorded from that cell. In these six myotubes, the average density of BI current was 17.5 ± 2.2 pA/pF and the maximum V_{err} was <5 mV. G_{max} for these six cells was 378 ± 40 nS/μF; the potential which activated half-maximal BI Ca^{2+} conductance (V_G) was 10 ± 3 mV (n = 6), and the steepness factor (k_G) was 4.2 ± 0.6 mV (n = 6). Steady HP was −80 mV. All test pulses were immediately preceded by a 1-s prepulse to −30 mV. The charge carrier was 10 mM Ca^{2+}.

current was clearly distinguished from the small, endogenous L-type Ca^{2+} current (I_{dys}) of dysgenic skeletal muscle (Adams and Beam, 1989) by its insensitivity to 1 μM (+)-PN 200–110, a potent dihydropyridine Ca^{2+} channel antagonist (n = 9; Fig. 2 *B*). A higher concentration (10 μM) of (+)-PN 200–110 also failed to inhibit the expressed BI Ca^{2+} current (n = 4; not shown). In 8 of 13 myotubes examined, (+)-PN 200–110 appeared to slightly potentiate the expressed BI current; however,

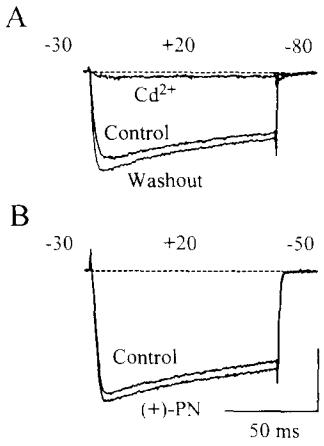

FIGURE 2. Effect of Ca^{2+} channel blockers on expressed BI Ca^{2+} currents. (*A*) BI current is reversibly blocked by Cd^{2+}. Maximal currents were recorded before (*Control*), during exposure to 100 μM Cd^{2+} and after washout (*Washout*). Cell BV65, C = 170 pF. (*B*) BI current is not inhibited by 1 μM (+)-PN 200–110. Maximal currents were recorded for a control solution exchange (*Control*) and during exposure to the drug ([+]-PN). Cell BV95, C = 210 pF. Pretest, test, and repolarization potentials are indicated above the current traces. Vertical calibration is 2.5 nA (*A*) and 2.0 nA (*B*).

this effect could not reliably be distinguished from current "run up" which was typically observed in our experiments.

To identify the mechanisms underlying inactivation of the BI current, we replaced all Ca^{2+} in the external solution with equimolar Ba^{2+}. For these experiments, 1 μM (+)-PN 200–110 was included in all external solutions to block any endogenous I_{dys} that might have been present. As shown in Fig. 3, substituting Ba^{2+} for Ca^{2+} as charge carrier substantially increased the peak amplitude of the expressed BI current (by 57 ± 7%; n = 8) without decreasing the rate of current inactivation. Substituting Ba^{2+} for Ca^{2+} also shifted the current-voltage relationship to more negative potentials by ~10 mV (n = 8; not shown).

To examine the ability of the expressed BI Ca^{2+} channels to generate intramembrane charge movement, we used a prepulse protocol (see Methods) that effectively isolates "immobilization-resistant" charge movements arising from high-voltage–activated, slowly-inactivating channels (Adams et al., 1990). The upper panel in Fig. 4*A* illustrates immobilization-resistant charge movement recorded from a BI-expressing dysgenic myotube after the addition of Cd^{2+} and La^{3+} to block all ionic Ca^{2+} currents. The immobilization-resistant charge movement in this cell was little different from that recorded from a typical control dysgenic myotube (Fig. 4*A*, *bottom*). In 16 BI-injected myotubes that expressed a peak Ca^{2+} current of 31 ± 4

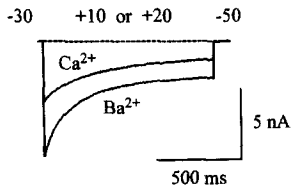

FIGURE 3. BI Ca^{2+} channels expressed in dysgenic myotubes do not exhibit Ca^{2+}-dependent inactivation. Shown are maximal BI currents recorded with either Ca^{2+} or Ba^{2+} as charge carrier. The time constants of inactivation (τ_h) for the currents shown were 278 ms with 10 mM Ca^{2+} in the external solution (peak current elicited by a test pulse to +20 mV) and 183 ms with 10 mM Ba^{2+} in the external solution (peak current elicited by a test pulse to +10 mV). Dysgenic myotube BV95, injected with pKCRCBI-2; C = 210 pF. Pretest, test, and repolarization potentials are indicated above the current traces. (+)-PN 200–110 (1 μM) was present in the external solutions throughout the experiment, in order to block endogenous I_{dys}.

pA/pF (G_{max} of 534 ± 51 μS/μF), the maximum, immobilization-resistant charge movement (Q_{max}) averaged 2.77 ± 0.21 nC/μF. For comparison, Q_{max} in noninjected dysgenic myotubes was 2.33 ± 0.24 nC/μF (n = 14). Possibly, some BI channel gating charge might have been immobilized by the prepulse to −30 mV that was a part of the voltage protocol used. However, as shown in Fig. 4 *B*, the BI Ca^{2+} current elicited with the prepulse protocol was indistinguishable from that elicited by a voltage step applied directly from the holding potential. Thus, it appears that expression of BI channels in dysgenic myotubes results in a high density of ionic Ca^{2+} channel current but very little immobilization-resistant charge movement.

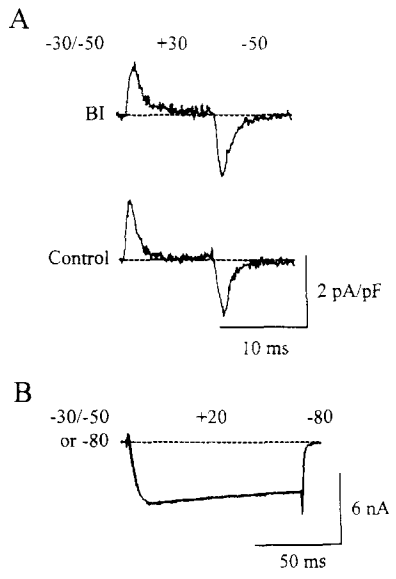

FIGURE 4. Expression of BI Ca^{2+} channels produces very little immobilization-resistant intramembrane charge movement. (*A*) Nonlinear charge movement in response to a strong depolarization in a dysgenic myotube expressing BI Ca^{2+} channels (*BI*) and in a noninjected dysgenic myotube (*Control*). The maximum Ca^{2+} current recorded from the myotube expressing BI channels was 38 pA/pF (elicited by a test pulse to +10 mV) and Q_{max} in this same myotube was 2.7 nC/μF (test pulse to +30 mV). $Q_{max\text{-}dys}$ recorded from the control dysgenic myotube was 2.5 nC/μF (test pulse to +30 mV). BI: dysgenic myotube BK13, injected with pKCRCBI-1; C = 280 pF. Control: noninjected dysgenic myotube BL71; C = 110 pF. Pretest, test, and repolarization potentials are indicated above the current traces. (*B*) Effect of the voltage protocol used to measure immobilization-resistant charge movement on ionic Ca^{2+} current through the BI channel. Shown are two superimposed current traces recorded using either the standard voltage protocol (test pulse to +20 mV delivered directly from the steady HP of −80 mV) or the prepulse protocol (a 1-s step to −30 mV followed by a 25-ms step to −50 mV preceded the test pulse to +20 mV). Cell BV93, C = 260 pF.

Dysgenic myotubes injected with BI channel cDNA were never observed to contract spontaneously, and evoked contractions were extremely rare. Out of more than 300 injected myotubes that were electrically stimulated via an extracellular pipette, only three myotubes displayed evoked contractions. In these three contracting myotubes, whole-cell patch clamp recordings subsequently revealed exceptionally high Ca^{2+} current densities (>50 pA/pF). Many other BI-injected myotubes which did not display evoked contractions expressed lower, but still quite significant densities of BI Ca^{2+} current (up to ~40 pA/pF). To identify the type of E-C coupling mediated in these rare instances by the expressed BI channels, we simultaneously recorded Ca^{2+} currents and monitored contractions in one of the few BI-expressing myotubes that displayed evoked contractions. Whole-cell Ca^{2+} currents were recorded from this cell

using a pipette solution in which the EGTA concentration was reduced from the usual 10 to 0.1 mM, a concentration which does not prevent contraction of normal myotubes or of dysgenic myotubes expressing the cardiac DHP receptor (Garcia, Tanabe, and Beam, 1994). In this particular myotube, a test depolarization to +20 mV elicited a large inward Ca^{2+} current and a corresponding contraction. When 100 μM Cd^{2+} was added to the external solution, the Ca^{2+} current was effectively blocked and the contractions were abolished (not shown). Washout of the Cd^{2+} completely restored the original density of the Ca^{2+} current and also restored the evoked contractions. This experiment indicates that in the rare instances where expression of the BI channel did restore E-C coupling, the coupling was of cardiac-type in that it required the entry of external Ca^{2+}.

DISCUSSION

The results presented here demonstrate several interesting features of the neuronal BI Ca^{2+} channel. When heterologously expressed in dysgenic myotubes, BI channels produce a high-density Ca^{2+} conductance which, surprisingly, almost never restores E-C coupling. Furthermore, the large Ca^{2+} conductance produced by the expressed BI channels is associated with a very low density of intramembrane charge movement, indicating that the BI channels exhibit a relatively large open probability. These findings are considered in more detail in the following paragraphs.

BI channels have previously been expressed heterologously in *Xenopus* oocytes (Mori et al., 1991; Sather et al., 1993). However, to the best of our knowledge, the present work constitutes the first report of heterologous expression of the neuronal BI Ca^{2+} channels in mammalian cells. In the previous studies, Mori et al. (1991) used 40 mM Ba^{2+} as charge carrier, whereas Sather et al. (1993) used either 2 or 40 mM Ba^{2+} as charge carrier. In contrast, in the present study we used either 10 mM Ba^{2+} or 10 mM Ca^{2+} as charge carrier because we wished to compare our results for the BI channels with those previously obtained for L-type channels expressed in dysgenic myotubes. Although differences in concentration and ionic species of charge carrier could easily affect the kinetics and voltage dependence of the expressed Ca^{2+} channels, it appears that BI channels expressed in dysgenic myotubes behave in a qualitatively similar fashion to BI channels expressed in *Xenopus* oocytes, despite the differences in experimental conditions used for the two systems. In both cell types, the BI currents activate within a few milliseconds, inactivate within a few hundred milliseconds, and are insensitive to DHP antagonists.

Perhaps the most significant result of the present study is that BI channels expressed in dysgenic myotubes produce a large Ca^{2+} conductance, but a low density of immobilization-resistant charge movement. If it is assumed that the immobilization-resistant charge movement observed in noninjected dysgenic myotubes is present as a contaminant of the charge movement recorded from BI-injected dysgenic myotubes, then the difference between the average Q_{max} of BI-injected myotubes (2.77 nC/μF) and noninjected dysgenic myotubes (2.33 nC/μF) provides an estimate of the charge movement (Q_{max}') attributable to the expressed BI channels. We and others have previously used the ratio G_{max}/Q_{max}' as a measure of the relative ability of expressed DHP receptors to function as L-type Ca^{2+} channels (Adams et al., 1990; Beam, Adams, Niidome, Numa, and Tanabe, 1992; Neely, Wei,

Olcese, Birnbaumer, and Stefani, 1993). In an earlier study (Adams et al., 1990), we found that dysgenic myotubes expressing cardiac L-type Ca^{2+} channels had a G_{max} (488 μS/μF) very similar to that found in the present study for dysgenic myotubes expressing BI channels (534 μS/μF; see Results). In contrast, for dysgenic myotubes expressing cardiac L-type channels, Q_{max}' was 8.9 nC/μF, a value much higher than ~0.44 nC/μF for Q_{max}' in dysgenic myotubes expressing BI channels. Consequently, the G_{max}/Q_{max}' ratio was much smaller for the cardiac L-type channels (55 nS/pC) than for the neuronal BI channels (>1,000 nS/pC). If $G_{max} = n\gamma P_0$ and $Q_{max}' = nq$, where n is the number of channels, γ is the single-channel conductance, P_0 is the maximum open probability, and q is the gating charge of a single channel, then $G_{max}/Q_{max}' = \gamma P_0/q$. Further, if the ratio γ/q is roughly constant, then G_{max}/Q_{max}' is directly proportional to P_0. In fact, γ appears to be smaller for BI than for cardiac L-type channels; with 110 mM barium as charge carrier, γ for BI channels expressed in *Xenopus* oocytes is ~16 pS, whereas γ for cardiac L-type channels is ~21 pS (Mori et al., 1991; Sather et al., 1993). Thus, the higher G_{max}/Q_{max}' ratio found for the BI channels suggests that they exhibit a considerably higher P_0 than cardiac L-type channels.

The actual P_0 for BI channels expressed in dysgenic myotubes can be estimated by the following calculation. If we assume that γ = 4 pS (with 10 mM Ca^{2+} as charge carrier, estimated using results presented in Gollasch, Hescheler, Quayle, Patlak, and Nelson, 1992), and q = 12 electronic charges (e^-; Schoppa, McCormack, Tanouye, and Sigworth, 1992; Zagotta, Hoshi, Dittman, and Aldrich, 1994), then multiplying 4×10^{-12} S/(12 e^-) by Avogadro's number (6.023×10^{23} e^-/mol) and Faraday's constant (96,487 C/mol) yields a conductance to charge ratio of $\sim 2.0 \times 10^6$ S/C; note that this value applies only to the special condition where the channels are always open, i.e., P_0 = 1.0, and furthermore this value is independent of the number of channels in the membrane. Multiplying 2.0×10^6 S/C by the estimated density of gating charge movement attributable to the expressed BI channels (0.44 nC/μF) yields a conductance to capacitance ratio of 880 S/F, which is ~1.65 times that calculated for BI channels in the present study (534 S/F; see Results). Thus, the P_0 of the expressed BI Ca^{2+} channels would have been ~0.6 in order to produce the measured conductance to capacitance ratio of 534 S/F; this value of P_0 is considerably higher than P_0 for L-type Ca^{2+} channels in cardiac cells (0.03; Lew et al., 1991), but is quite comparable to P_0 for N-type channels in neurons of the frog sympathetic ganglion (0.5; Delcour and Tsien, 1993). It is interesting to speculate that a relatively high P_0 may be a general feature of neuronal, non-L-type Ca^{2+} channels, whereas a relatively low P_0 may be a general feature of muscle L-type Ca^{2+} channels.

Our present results demonstrate that BI channels expressed in dysgenic myotubes do not exhibit Ca^{2+}-dependent inactivation (Fig. 3). This finding is in agreement with that of Sather et al. (1993) for BI channels expressed in cell-attached patches from *Xenopus* oocytes. The absence of Ca^{2+}-dependent inactivation may be an intrinsic property of the BI channel protein (the α1 subunit), or alternatively may reflect the absence in both dysgenic myotubes and *Xenopus* oocytes of ancillary proteins required for Ca^{2+}-dependent inactivation. The latter possibility is suggested by previous results (Tanabe, Mikami, Numa, and Beam, 1990) demonstrating that cardiac L-type Ca^{2+} channels, which display prominent Ca^{2+}-dependent inactivation in their native

cellular environment (cardiac myocytes), do not exhibit prominent Ca^{2+}-dependent inactivation when heterologously expressed in dysgenic myotubes.

The dysgenic myotube expression system makes it possible to test the ability of different types of Ca^{2+} channels to mediate E-C coupling. We found that myotubes expressing BI Ca^{2+} channels almost never showed restored E-C coupling. Thus, only ~1% of dysgenic myotubes injected with BI channel cDNA displayed evoked contractions, whereas E-C coupling was restored in ~17% of dysgenic myotubes injected with cDNA encoding the cardiac L-type Ca^{2+} channel (Tanabe et al., 1990). This difference in restored E-C coupling is surprising because the average current densities are comparable in dysgenic myotubes expressing the BI (~30 pA/pF) and cardiac (~28 pA/pF) Ca^{2+} channels. It seems noteworthy that heterologous expression of neuronal BIII (N-type) Ca^{2+} channels also fails to restore E-C coupling to dysgenic myotubes (Fujita et al., 1993). Why do cardiac and skeletal muscle L-type Ca^{2+} channels usually restore E-C coupling in dysgenic myotubes, whereas neuronal BI and BIII Ca^{2+} channels do not? One possibility, suggested by the low densities of intramembrane charge movement recorded from myotubes expressing the BI channels, is that so few BI channels are present in the membrane that even if they are directly apposed to the Ca^{2+} release channels of the sarcoplasmic reticulum (SR) they cannot activate enough of the release channels to cause contraction. Alternatively, the expressed BI and BIII channels may not become localized sufficiently close to the SR release channels of the sarcoplasmic reticulum (SR), so that even significant entry of Ca^{2+} via these neuronal channels is unable to trigger SR Ca^{2+} release effectively. Our present results with the BI channel are consistent with the possibility that these channels become targetted to different regions of dysgenic myotubes than exogenously expressed L-type Ca^{2+} channels. Future studies will be necessary to determine the subcellular localization of different types of Ca^{2+} channels and to elucidate the mechanisms underlying channel localization.

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