Alterations in dihydropyridine receptors in dystrophin-deficient cardiac muscle

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Woolf, Peter J., Sai Lu, Renee Cornford-Nairn, Michael Watson, Xiao-Hui Xiao, Sean M. Holroyd, Lindsay Brown, and Andrew J. Hoey. Alterations in dihydropyridine receptors in dystrophin-deficient cardiac muscle. Am J Physiol Heart Circ Physiol 290: H2439–H2445, 2006. First published January 13, 2006; doi:10.1152/ajpheart.00844.2005.—The deficiency of dystrophin, a critical membrane stabilizing protein, in the mdx mouse causes an elevation in intracellular calcium in myocytes. One mechanism that could elicit increases in intracellular calcium is enhanced influx via the L-type calcium channels. This study investigated the effects of the dihydropyridines BAY K 8644 and nifedipine and alterations in dihydropyridine receptors in dystrophin-deficient mdx hearts. A lower force of contraction and a reduced potency of extracellular calcium (P < 0.05) were evident in mdx left atria. The dihydropyridine agonist BAY K 8644 and antagonist nifedipine had 2.7- and 1.9-fold lower potencies in contracting left atria (P < 0.05). This corresponded with a 2.0-fold reduction in dihydropyridine receptor affinity evident from radioligand binding studies of mdx ventricular homogenates (P < 0.05). Increased ventricular dihydropyridine receptor protein was evident from both radioligand binding studies and Western blot analysis and was accompanied by increased mRNA levels (P < 0.05). Patch-clamp studies in isolated ventricular myocytes showed no change in L-type calcium current density but revealed delayed channel inactivation (P < 0.05). This study indicates that a deficiency of dystrophin leads to changes in dihydropyridine receptors and L-type calcium channel properties that may contribute to enhanced calcium influx. Increased influx is a potential mechanism for the calcium overload observed in dystrophin-deficient cardiac muscle.

Duchenne muscular dystrophy; calcium channels; heart

DYSTROPHIN IS AN INTEGRAL subsarcolemmal protein essential for stabilizing both the dystrophin-associated glycoprotein complex (12) and the membrane of cardiac myocytes during contractions (7). The absence of dystrophin is the primary molecular basis of Duchenne muscular dystrophy (DMD), a fatal X-linked neuromuscular disorder affecting 1 in 3,500 live male births (11). Cardiac dysfunction is observed frequently, with cardiomyopathy shortening the lifespan of a significant proportion of boys with DMD (5, 10, 15, 16, 23). In addition to congestive heart failure, other cardiac manifestations of DMD include altered heart rate variability, arrhythmias, conduction defects, and fibrosis (17). Similarly, a reduced dystrophin expression is the primary basis of congestive heart failure in X-linked cardiomyopathy and Becker’s muscular dystrophy (6), with cardiac transplantation being undertaken as a long-term treatment option.

The dystrophin-deficient mdx mouse arose as a spontaneous mutation from a colony of C57BL10/SvSn (C57) mice and has been widely used as an animal model of DMD for research investigating skeletal muscle dysfunction. However, the cardiovascular manifestations caused by dystrophin deficiency in the mdx mouse have not been reported extensively. We have previously reported altered responsiveness to α-adrenoceptor and muscarinic receptor stimulation in young (12–14 wk old) mice as well as a reduced efficacy and potency of calcium (20). In old mdx males (12 mo), a lowered efficacy and potency of l-isoprenaline and reduced β1-adrenoceptor affinity is evident (19).

Isolated cardiac myocytes (1, 13) and skeletal muscles (31, 32) from mdx mice and patients with DMD (3) exhibit elevated intracellular calcium. This calcium overload can lead to decreased myocyte function and myocyte necrosis (4), resulting from calcium-dependent proteolysis (30). Furthermore, mdx mice have been shown to exhibit dysfunctional calcium handling in both skeletal (28) and cardiac (21) myocytes. Cardiac calcium overload could potentially be due to a leaky cell membrane as postulated in skeletal muscle (31) or may result from alterations in influx or efflux, intracellular calcium handling, sarcoplasmic reticulum storage, release, sequestration, or a combination of these mechanisms.

The major source of calcium influx across the cardiac cell membrane is the L-type calcium channel. This channel consists of a complex of five subunits, with the α1-subunit being the channel-forming, voltage-sensing component and containing the binding site for dihydropyridine (DHP) compounds such as the antagonist nifedipine and agonist BAY K 8644. This α1-subunit, also commonly known as the DHP receptor (DHRPR), is a defining feature of cardiac L-type calcium channels and is localized within the cardiac T-tubule system, which is where dystrophin is also located (25). Thus the primary aim of this study was to examine the consequences of dystrophin deficiency on the DHPRs as a marker of L-type calcium channels regulating calcium influx.

MATERIALS AND METHODS

All experimental protocols were approved by the University of Southern Queensland Animal Ethics committee under the guidelines of the National Health and Medical Research Council of Australia, which conforms to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Male muscular dystrophy X-linked (mdx) mice and their control C57BL10/SvSn (C57) were

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used at 12–14 wk of age. Mice were fed standard mouse chow ad libitum and maintained on a 12-h:12-h light-dark cycle.

**Tissue bath experiments.** Animals were anesthetized by excess CO2 inhalation and euthanized by exsanguination before excision of their hearts. Left atria were dissected free in cold, carbogenated (95% O2:5% CO2) Tyrode physiological salt solution (TPSS) containing (in mM) 136.9 NaCl, 5.4 KCl, 1.05 MgCl2·H2O, 0.42 NaH2PO4·2H2O, 22.6 NaHCO3, 1.8 CaCl2·2H2O, 5.5 glucose, 0.28 ascorbic acid, and 0.05 Na2EDTA. A stainless steel hook was placed in one end of each atrium to connect that atrium to the tissue holder. A silk thread was tied to the other end of the atrium to connect it to a force transducer (FT-102, CB Sciences, Milford, MA) to measure the force of contraction. Atrial tissues were subsequently suspended in warm (35 ± 1°C), carbogenated TPSS in tissue baths. Data were recorded via a PowerLab system (AD Instruments) using Chart 3.5.6. Left atria were field stimulated (AMPI Master 8 stimulator; 1 Hz, 5-ms duration, 20% above threshold) and maintained under optimal preload. Tissues were allowed 45 min to equilibrate with repetitive washes. At the completion of all experiments, the atria were removed, blotted, and weighed.

There was no significant difference between the blotted masses of the atria taken from mdx and C57, so all force data are reported in millinewtons.

**Nifedipine and BAY K 8644 concentration response curves.** Concentration-response curves were generated to calcium chloride to determine the maximum force of contraction attainable by the tissue. The tissues were then washed repetitively over 30 min to return the contractility to normal, and a concentration-response curve measuring the negative inotropic effects of the DHP antagonist nifedipine was generated. To avoid indirect receptor-mediated effects of endogenous norepinephrine possibly released by BAY K 8644, tissues were generated. To avoid indirect receptor-mediated effects of endogenous norepinephrine possibly released by BAY K 8644, tissues were generated. To avoid indirect receptor-mediated effects of endogenous norepinephrine possibly released by BAY K 8644, tissues were generated. To avoid indirect receptor-mediated effects of endogenous norepinephrine possibly released by BAY K 8644, tissues were generated. To avoid indirect receptor-mediated effects of endogenous norepinephrine possibly released by BAY K 8644, tissues were generated.

**Western blot analysis.** Total protein was extracted by homogenizing frozen ventricles in lysis buffer (4% SDS, 125 mM Tris·HCl (pH 6.8), 40% glycerol, and 50 mM DTT), containing Protease Inhibitor Cocktail (Sigma) and 0.5 mM phenylmethylsulfonylfluoride. Proteins (60 µg) were separated on SDS-PAGE using a NuPAGE precast 4–12% Bis-Tris polyacrylamide gel and MOPS SDS running buffer (Invitrogen). After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Amersham). Transfer was confirmed by staining the membrane with Ponceau S stain (0.1% Ponceau S in 5% acetic acid). For detection of the α1c-subunit, the top half of the membrane was blocked in 1× Tris-buffered saline-Tween 20 containing 5% skim milk powder and 0.05% Tween 20. This was followed by incubation with a 1:200 dilution of anti-α1c antibody (Sigma) at 4°C overnight and afterward for 1 h in a 1:10,000 dilution of peroxidase-conjugated antibody (goat anti-rabbit polyclonal, Sigma). For detection of α-actin, the lower half of the membrane was blocked in 1× Tris-buffered saline-Tween 20 containing 5% BSA and 0.1% Tween 20. This was followed by incubation with a 1:1000 dilution of anti-α-actin monoclonal antibody (Clone AC-40, Sigma) at room temperature for 90 min and afterward for 1 h in a 1:6,000 dilution of peroxidase-conjugated antibody (rabbit anti-mouse polyclonal, DakoCytomation). The immunoreactive bands on both Western blots were visualized by using ECL Plus and Hyperfilm ECL (Amersham). The Western blots were analyzed using Scion Image. The α1c protein was normalized to α-actin to account for possible loading comparisons.

**Patch clamp.** Animals were anesthetized by pentobarbital sodium (70 mg/kg) and euthanized by exsanguination before excision of their hearts. The aorta was cannulated, and the hearts were suspended on a Langendorff system for perfusion with calcium free solution containing (in mM) 100 NaCl, 5 KCl, 1.2 KH2PO4, 5 MgSO4, 50 taurine, 10 HEPES, 20 glucose, and 10 creatine at 37°C for 5 min, followed by perfusion with collagenase type II solution (Worthington), 1% BSA (Sigma), and 0.08 mg/ml protease (Sigma) for 12 to 18 min. The ventricles were then removed and crudely dissociated with scissors before the addition of cell Tyrode solution containing (in mM) 100 NaCl, 5 KCl, 1.2 KH2PO4, 5 MgSO4, 50 taurine, 10 HEPES, 20 glucose, and 10 creatine with 0.4 mM CaCl2. After 10 min, this solution was aspirated and replaced sequentially with solution containing 0.8 and, finally, 1.2 mM CaCl2 to produce calcium-tolerant myocytes.

**RT-PCR.** Animals were anesthetized with pentobarbital sodium and euthanized by exsanguination before hearts were excised. The ventricles and left atria were dissected free in TPSS and placed in RNAlater (Ambion) RNA stabilization reagent for storage at −20°C. RNA extraction was performed with the QIAGEN RNaseasy Mini Kit, utilizing no more than 30 mg of tissue per sample obtained from the apex of the left ventricle or the whole left atria. The tissue was lysed using a polytron rotor-stator homogeniser in proprietary buffer containing guanidium thiocyanate to liberate RNA before the extraction procedure. RNA was isolated via binding to a silica-gel-based membrane during a number of centrifugation procedures as per the manufacturer’s instructions.

RT-PCR was performed by the two-tube method using QIAGEN Omniscript Reverse Transcriptase and QIAGEN HotStarTaq DNA Polymerase. Tubes containing the reaction mixture were incubated in a Corbett Research PC-960C thermocycler. α1c-Subunit primer (0.2 µM) (TCC AGT TTA TAC TAC TTT GGC TGG TTG T sense; and ACT GAG GGC TCA TGT TTT GG antisense) or GAPDH primer (0.2 µM) (TTA GCA CCC CTG GCC AAG G sense; and CTT ACT CCT TGG AGG CCA TG antisense) and HotStarTaq DNA Polymerase were used. PCR product was visualized after gel electrophoresis by ethidium bromide staining and UV transillumination in an Alphalmager 2200 MultiImage cabinet. Spot densitometry was performed by using AlphaEase, and the ratio of DHPR band intensity to GAPDH band intensity was calculated for each sample to allow a semiquantitative comparison.

**Radioligand binding.** Ventricular tissues were snap frozen in liquid nitrogen and stored at −70°C. For each experiment two ventricles were pooled to obtain sufficient membrane for five points on a saturation curve. Two ventricles were homogenized at 24,000 rpm by a Heidolph DIAx 600 after being thawed in Tris incubation solution containing (in mM) 50 Tris·HCl, 5 EGTA, 1 EDTA, 4 MgCl2, 1 ascorbic acid, and 50 Trizma base (pH 7.4, adjusted with NaOH). The homogenate was centrifuged at 1,000 g, and the pellet was discarded.

The supernatant was centrifuged at 100,000g and C57, so all force data are reported in millinewtons.

**RT-PCR.** Animals were anesthetized with pentobarbital sodium (70 mg/kg) and euthanized by exsanguination before excision of their hearts. The aorta was cannulated, and the hearts were suspended on a Langendorff system for perfusion with calcium free solution containing (in mM) 100 NaCl, 5 KCl, 1.2 KH2PO4, 5 MgSO4, 50 taunine, 10 HEPES, 20 glucose, and 10 creatine at 37°C for 5 min, followed by perfusion with collagenase type II solution (Worthington), 1% BSA (Sigma), and 0.08 mg/ml protease (Sigma) for 12 to 18 min. The ventricles were then removed and crudely dissociated with scissors before the addition of cell Tyrode solution containing (in mM) 100 NaCl, 5 KCl, 1.2 KH2PO4, 5 MgSO4, 50 taunine, 10 HEPES, 20 glucose, and 10 creatine with 0.4 mM CaCl2. After 10 min, this solution was aspirated and replaced sequentially with solution containing 0.8 and, finally, 1.2 mM CaCl2 to produce calcium-tolerant myocytes.

**Cells were placed in a 0.5-mL perfusion chamber, allowed to settle for 5 min, and then perfused at room temperature with oxygenated extracellular solution containing (in mM) 50 NaCl, 3 MgCl2, 1.8 CaCl2, 3 KCl, 90 tetraethylammonium-Cl, 7.7 glucose, and 10 HEPES
(pH 7.4). Cells were patched by using fire-polished electrodes (1.5–2.5 MΩ), pulled from filamented borosilicate glass (World Precision Instruments), and filled with electrode solution containing (in mM) 135 CsF, 10 NaCl, 10 HEPES, 10 EGTA, 7.7 glucose, and 2 MgATP (pH 7.2). Cell recordings were obtained by using a HEKA EPC-9. Cell capacitance was recorded, and the cells were then stepped from a holding potential of −70 to −40 mV for 40 ms to inactivate the sodium current. Cells were then stepped in 10-mV increments from −40 up to +60 mV for 200 ms durations at 0.2 Hz. Initial experiments confirmed recordings of L-type calcium currents by verifying a lack of effect by 1 μM tetrodotoxin and a concentration-dependent inhibition of L-type calcium current by verapamil (1–10 μM) and cadmium (50 μM). Current density was calculated by dividing current amplitude by cell capacitance (in pA/pF).

Reagents and data analysis. All reagents and pharmacological compounds were purchased from Sigma-Aldrich, except where otherwise indicated. Data are presented as means ± SE of the number (n) of experiments. Data were compared by using Student’s two-tailed t-test, and a P < 0.05 was considered statistically significant.

RESULTS

Before conducting the concentration-response curves to the DHPs, the mdx left atria had a lower basal force of contraction (1.50 ± 0.17 mN) compared with C57 (1.91 ± 0.18 mN; P < 0.05). A subsequent concentration-response curve to calcium chloride (Fig. 1A) revealed increases in contractile force; however, when normalized to the basal force of contraction, the percent change in both tissues was found to be similar. Of interest was the potency (EC50) values calculated from this data that showed a significant difference in mdx compared with C57 (mdx, 3.40 ± 0.08 × 10−3 M; and C57, 3.15 ± 0.10 × 10−3 M; P < 0.05).

Similarly, nifedipine reduced the force of contraction in left atria from both mdx and C57 but was significantly less potent in left atria from mdx (EC50 values; mdx, 3.47 ± 0.55 × 10−8 M; and C57, 1.78 ± 0.40 × 10−8 M; P < 0.05, Fig. 1A). A strain potency ratio for nifedipine was calculated by dividing the EC50 of nifedipine in mdx by the EC50 of nifedipine in C57 to give a value of 1.9. BAY K 8644 elicited a strong positive inotropic effect in both strains, producing a similar efficacy to calcium. Although the efficacy (as a percentage of calcium chloride) was not different between the two strains, a reduced potency (EC50 values; mdx, 7.94 ± 0.55 × 10−7 M; and C57, 2.95 ± 0.34 × 10−7 M; P < 0.05; Fig. 1B) was again evident in mdx. The strain potency ratio for BAY K 8644 was 2.7, which is similar to the ratio obtained for nifedipine. The solvent ethanol elicited a weak negative inotropic effect at the maximum solvent concentration, but this was completely overcome by the pronounced positive inotropic effect of BAY K 8644.

Radioligand binding experiments revealed a significantly greater number of specific binding sites (Bmax) of DHPRs in mdx (Bmax, 99.2 ± 7.0 fmol/mg) compared with C57 (Bmax, 74.5 ± 9.4 fmol/mg; P < 0.05; Fig. 2A) with a strain ratio (Bmax mdx/Bmax C57) of 1.3. The affinity of [3H]PN 200–110 was also reduced significantly in mdx (Kd, 0.3677 ± 0.08 nM) compared with C57 (Kd, 0.1789 ± 0.08 nM) (P < 0.05; Fig. 2B). The strain potency ratio (Kd mdx/Kd C57) was 2.0, a similar ratio to that observed in the functional tissue bath studies utilizing nifedipine and BAY K 8644.

To confirm the result of increased DHPR protein observed in the radioligand binding study, Western blot analysis was also conducted on ventricular myocardium. Again, this revealed increased DHPR protein in the mdx (Fig. 2C). This was supported by the observation that ventricular DHPR mRNA was significantly higher (P < 0.005) in mdx compared with C57 (Fig. 2D) when corrected for the amount of total mRNA by using the GAPDH samples. The strain ratio (band intensity mdx/band intensity C57) for ventricular mRNA was 2.3. The left atrial DHPR mRNA was also higher in mdx compared with C57, although this did not reach statistical significance (P = 0.07). The strain ratio for atrial mRNA for DHPRs was 2.0.

The ubiquitous β-actin was also used as a housekeeper gene (data not shown) and produced qualitatively similar data to GAPDH normalization.

Patch-clamp experiments were then conducted to determine whether the increased DHPR expression resulted in a larger current amplitude. The currents were not inhibited by tetrodotoxin but were dose-dependently inhibited by verapamil and cadmium, confirming the appropriateness of the experimental conditions (data not shown). There was no significant difference in whole cell membrane capacitance (mdx, 161 ± 10 pF; and C57, 147 ± 13 pF; n = 10) and no significant difference...
in peak current density at a test potential of 0 mV (mdx, 4.67 ± 0.39 pA/pF; and C57, 5.45 ± 0.58 pA/pF). However, further analysis revealed that the time to 90% inactivation of the L-type current was increased in mdx myocytes compared with C57 myocytes (P < 0.05; Fig. 3).

**DISCUSSION**

This study is the first to measure the properties of DHPRs in dystrophin-deficient myocardium and shows a reduced potency to both a DHPR agonist and antagonist, reflected by reduced receptor affinity, upregulation of DHPR mRNA and protein, and delayed inactivation of the L-type calcium current.

Alterations in receptor regulation are a commonly observed phenomenon in cardiovascular disease states. Such alterations are generally evidenced initially by impaired potencies or efficacies to agonists that reflect changes in receptor stimulus; however, this study showed a similar difference in potency with the DHPR antagonist nifedipine. This potency difference can be explained by a lowered receptor affinity (higher Kd), as observed in the radioligand binding studies that utilized antagonists and thus not dependent on receptor stimulus and agonist efficacy.

A possible mechanism that could affect the affinity of DHPRs is a change in the proportion of channels in the inactivated state that could be reflected by a change in the cell membrane potential, with depolarization of the cardiac resting membrane potential causing an increase in DHPR affinity and thus presumably hyperpolarization, causing a decrease in DHPR affinity (2). However, microelectrode studies in left atria of mdx and C57 mice, utilizing the same conditions as those in the tissue bath studies, did not show any difference in resting membrane potential with or without a calcium channel antagonist present (data not shown), eliminating this as a basis for the difference in DHPR affinity in the tissue bath studies. Furthermore, the difference in receptor affinity was maintained in the radioligand binding studies, where the influence of the membrane potential is eliminated. This provides clear evidence that a change in receptor affinity is not due to changes in membrane potential.

Another influence affecting DHPR affinity is the concentration of free calcium. Using [3H]PN 200–110 in radioligand binding studies, Peterson and Catterall (26) showed that divalent ions cause a biphasic effect on the affinity of DHPRs, with a low free calcium causing an increase in affinity, whereas a high free calcium causes a significant decrease in affinity. It is, therefore, highly feasible that the calcium overload observed in mdx myocytes is a mediator responsible for the lowered affinity state of the DHPRs observed in the atrial contractility studies. However, in DHPR radioligand binding studies, calcium is not added to the incubation buffer, and chelators, such as EGTA, are also present. This then eliminates any acute effects of calcium on modifying the affinity state of the DHPRs within such studies. Given that a lowered affinity for [3H]PN 200–110 was still observed, this may suggest that chronic in vivo
elevation of intracellular calcium may cause a more rigid conformational change in DHPRs, which is subsequently maintained in isolated membranes.

Alternatively, a disruption in DHPR localization due to dystrophin deficiency may be possible. DHPRs are located in the transverse tubules (T tubules) of the myocyte that is also the major site where cardiac dystrophin is located (25, 29). Furthermore, dystrophin has been suggested to play a role in anchoring or modulating the activity of cardiac DHPRs (24), among other channels (8). Therefore, the deficiency of dystrophin could result in a direct defect in the DHPR that in turn may contribute to the observed reduction of potency in mdx cardiac tissues and a compensatory increase in DHPRs. It is highly probable that an increase in intracellular calcium is the cause of the elevated DHPRs, as Davidoff et al. (8) reported that incubation of adult rat ventricular myocytes in high extracellular calcium (4.8 mM) showed remarkably similar changes to those reported in the current study, including elevated DHPR mRNA levels of 1.5-fold and DHPR density of 2.0-fold.

Clearly, if an elevation of DHPRs is evident, then an alteration in the L-type calcium current could be expected. This current study, using 12- to 14-wk-old mice, showed no change in current density between mdx and C57, confirming previous findings by Alloatti et al. (1), who utilized mdx mice (of an unstated age), and Sadeghi et al. (27), who utilized cultured neonatal cardiac myocytes from 1- to 4-day-old mdx mice. The basis for the lack of change in current density has not been ascertained to date and requires further investigation. Possible mechanisms could be either an uncoupling and/or mislocalization of additional DHPR (α1c) subunits from the remaining L-type calcium channel subunits or an elevation in intracellular calcium reducing the driving force for calcium influx (9).

Although no change in current density was evident, a significant delay in inactivation of the L-type calcium current was observed, further confirming similar findings obtained by Davidoff et al. (8). Inactivation of L-type calcium channels is controlled by voltage and calcium-dependent mechanisms (18). A previous study,
using neonatal mdx myocytes and barium as the charge carrier instead of calcium because it does not contribute effectively in mediating ion-dependent inactivation, showed that dystrophin deficiency alters L-type calcium channel inactivation by a shift in the voltage dependence of activation. The current study used calcium as the charge carrier and, as such, allows a contribution of calcium-dependent inactivation mechanisms. Such inactivation will thus be affected by alterations in calcium-handling proteins such as sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) with a reduced SERCA activity having been shown to slow calcium-dependent inactivation (22). The mdx mouse has been reported to have reduced expression of SERCA, suggesting that calcium-dependent inactivation may also be slowed in mdx cardiac myocytes. Finally, the lack of dystrophin may also directly alter calcium-dependent L-type channel inactivation because dystrophin may bind directly to calcium channels to affect inactivation (27). The result of the delay in inactivation could be a contributor to the further development of calcium overload in mdx cardiac myocytes, which, in turn, would directly affect contractility.

Distinct differences in contractility were observed with a significantly lower force of contraction from left atria of mdx compared with C57 mice evident before the addition of drugs in all of the functional experiments undertaken. This impaired capacity to generate force remained despite elevated extracellular calcium as previously reported (19, 20). Interestingly, the potency of calcium is also lowered when conducting a concentration-response curve to extracellular calcium. This reduction in potency to calcium and reduced capacity to generate force are indicative of a reduced sensitivity of the contractile proteins to calcium. One possible explanation for this is that chronically elevated intracellular calcium, such as that observed in dystrophic muscles, could cause a significant reduction in myofilament sensitivity (14).

In conclusion, dystrophin is essential for normal regulation of the L-type calcium current, DHPRs, and cardiac contractility. The delayed inactivation of the L-type calcium current is a likely contributor to the elevation in intracellular calcium in the dystrophic heart, although other mechanisms, such as calcium sequestration or calcium leak from the sarcoplasmic reticulum, may also be involved. The elevation in intracellular calcium is likely to contribute to the incidence of arrhythmias in DMD as well as the altered cardiac contractility.

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GRANTS

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