T-Type Ca\(^{2+}\) Channels Are Abnormal in Genetically Determined Cardiomyopathic Hamster Hearts

Luyi Sen, Thomas W. Smith

Abstract  Although there is substantial evidence of abnormal Ca\(^{2+}\) homeostasis in heart cells of the cardiomyopathic Syrian hamster (Bio 14.6 strain), the mechanism by which these myocytes become Ca\(^{2+}\)-overloaded is not known. To elucidate the role of voltage-sensitive Ca\(^{2+}\) channels in the pathogenesis of myopathy, whole-cell Ca\(^{2+}\) currents were measured in myopathic and normal control cardiac myocytes. These studies demonstrate the presence of two voltage-sensitive Ca\(^{2+}\) channel types in ventricular myocytes isolated from 200- to 300-day-old cardiomyopathic and age-matched normal hamsters. The two Ca\(^{2+}\) channel types were identified by their unitary conductance properties and pharmacological sensitivities. Both L-type and T-type Ca\(^{2+}\) channels were present in cardiomyopathic and normal cells. Current density through L-type Ca\(^{2+}\) channels was the same in cardiomyopathic and normal control myocytes. However, the mean current density of T-type Ca\(^{2+}\) channels in cardiomyopathic cells was significantly higher than in normal cells (myopathic, 12.3±1.8 pA/pF; normal, 5.8±1.1 pA/pF; n=8; P<.01). The T-type Ca\(^{2+}\) current in cardiomyopathic myocytes was activated and inactivated at a high rate. These findings demonstrate no abnormality of the dihydropyridine-sensitive voltage-dependent L-type Ca\(^{2+}\) channel. In contrast, the observed abnormalities in T-type Ca\(^{2+}\) channel function in cardiomyopathic hamster myocytes suggest that this alteration may be related to the pathogenesis of Ca\(^{2+}\) overload and the arrhythmias in this genetically determined form of cardiomyopathy. (Circ Res. 1994;75:149-155.)

Key Words • T-type Ca\(^{2+}\) channel • L-type Ca\(^{2+}\) channel • cardiac myocytes • cardiomyopathic hamsters

The cardiomyopathic Syrian hamster is known to develop a genetically determined cardiomyopathy, with development of progressive and ultimately fatal congestive heart failure. This model has been used extensively for studies of fundamental cellular abnormalities in heart failure. The myocardium of the cardiomyopathic hamster (Bio 14.6 strain) shows evidence of intracellular Ca\(^{2+}\) overload, and it has been suggested that excess free intracellular Ca\(^{2+}\) plays an important role in the pathogenesis of this disease. Some studies have reported that an augmented number of dihydropyridine binding sites accompanied an increased voltage-dependent Ca\(^{2+}\) accumulation in the cardiomyopathic hamster heart may be responsible for the pathological alterations, but other laboratories have reported conflicting findings. It has long been postulated that increased influx of Ca\(^{2+}\) via voltage-sensitive Ca\(^{2+}\) channels may cause Ca\(^{2+}\) overload. However, the underlying mechanism by which heart cells of the cardiomyopathic hamster become Ca\(^{2+}\)-overloaded is unknown.

To elucidate the role of voltage-sensitive Ca\(^{2+}\) channels in the pathogenesis of cardiomyopathy, whole-cell Ca\(^{2+}\) currents were measured in cardiomyopathic (Bio 14.6 strain) and normal control (FIB) cardiac myocytes. We report here that both L- and T-type Ca\(^{2+}\) channels were present in ventricular myocytes isolated from 200- to 300-day-old cardiomyopathic and age-matched normal hamsters. The mean current density of T-type Ca\(^{2+}\) channels in cardiomyopathic cells was significantly (more than twofold) higher than in normal cells, whereas the current density through L-type Ca\(^{2+}\) channels was the same in both groups. Thus, Ca\(^{2+}\) entry into myocytes via T-type Ca\(^{2+}\) current may play an important pathogenetic role in this cardiomyopathy.

Materials and Methods

Cell Preparation

In the present study, we established methods for isolating cardiac myocytes from 8-month-old cardiomyopathic hamsters and age- and sex-matched normal control hamsters. Eight-month-old Bio 14.6 cardiomyopathic and FIB control hamsters were obtained from Bio-breeders. Normal and cardiomyopathic hamsters were anesthetized with ether, and the hearts were rapidly isolated. After cannulation of the aorta, hearts were perfused with oxygenated (37°C) Krebs-Henseleit (K-H) bicarbonate-buffered solution (pH 7.30) containing (mmol/L) NaCl 118, KCl 4.7, CaCl\(_2\) 0.6, MgSO\(_4\) 1.20, KH\(_2\)PO\(_4\) 1.20, NaHCO\(_3\) 25, and glucose 15. Hearts were perfused at 5 mL/min for 6-8 minutes until completely cleared of blood. Hearts were then perfused with the same solution but without CaCl\(_2\) for 5 minutes. Collagenase (0.03%) was added and recirculated for an additional 25 minutes. The ventricular muscle was removed from the perfusion apparatus, cut into 3-mm\(^3\) pieces, and placed in a 10-mL flask with 0.03% collagenase, 0.015% hyaluronidase, 0.0015% trypsin, 0.0015% deoxyribonuclease, and 1 mmol/L CaCl\(_2\) in K-H buffer. The
flask was shaken at 37°C for 15 minutes, and tissue pieces were transferred to Ca2+-free enzyme buffer with 2.0% bovine serum albumin. Tissue pieces were mechanically dissociated by gently triturating 10 to 15 times with a 5-mL pipette (tip diameter, 3 mm). The isolated myocytes were filtered through Nitex mesh and collected in a centrifuge tube. Cells were centrifuged two times at 500 rpm for 1 minute in Ca2+-free K-H buffer to remove dissociation enzymes. The isolated myocytes were resuspended in 0.6 mmol/L Ca2+ solution. The cells were permitted to attach to 12-mm circular glass coverslips coated with collagen. The viability of isolated cardiac myocytes from cardiomyopathic and normal hamsters was assessed by adding an aliquot of cell suspension to an equal volume of physiological buffer containing 0.4% Trypan blue. Cells were considered viable if they were rod-shaped, had clear cross striations, and excluded Trypan blue. Disaggregation of myocytes from a single heart produced ~5 × 10⁶ cells from either cardiomyopathic or normal hamsters. Although there was some variability in the number of viable cells produced per experiment, ~80% of the cells harvested from normal hamster hearts and 75% of those from cardiomyopathic hamsters were rod-shaped after a 5-minute incubation in physiological buffer containing 0.6 mmol/L [Ca2+]. The morphology by phase-contrast microscopy of isolated cardiac myocytes from cardiomyopathic and normal hamsters was indistinguishable. We have previously used myocardial cells isolated by use of this same procedure for the study of Ca2+ homeostasis and contractile function.11

**Tight-Seal Whole-Cell Recording**

The whole-cell voltage clamp was carried out by the suction pipette method.12,13 The pipettes were made of Borosil glass (Rochester Scientific), pulled by a two-stage puller (PE-2, Narishige), and heat-polished before use. An Axon patch amplifier (Axon Instruments, Inc) was used for the voltage-clamp experiments. Protocol generation and data acquisition were controlled by an IBM/AT and a 12-bit analog-to-digital and digital-to-analog converter (Axon Instruments) with the use of pCLAMP software (Axon Instruments). Data were filtered at 3 kHz by a three-pole Bessel filter incorporated in the Axopatch-1C amplifier, digitized on-line at a sampling interval of 0.3 millisecond, and stored on floppy diskettes for off-line analysis. The stray capacitance was compensated by the capacitance compensation circuit of the Axopatch-1C amplifier. In experiments kept for analysis, the series resistance remaining after compensation did not exceed 2.5 MΩ. Linear components of capacitance and leak currents have been subtracted digitally from all recordings shown by using scaled current from signal-averaged small pulses that elicited noionic current. The cell capacitance was estimated by dividing the area under a capacitative transient by the voltage step used to induce it (from ~80 to ~75 mV). The average cell capacitance was 148 ± 32 pF for normal cells (n = 58) and was significantly larger at 201 ± 46 pF for cardiomyopathic cells (n = 58, P < .01). All recordings were obtained at room temperature (22°C to 24°C). Whole-cell Ca2+ current recordings were obtained with the following solutions: the external solution contained (mmol/L) barium acetate 20, tetraethylammonium aspartate 135, and HEPES 10, pH 7.5; the internal solution contained (mmol/L) cesium aspartate 135, Cs2EGTA 10, HEPES 10, ATP 4, and MgCl2 5, pH 7.5. For Na+ current measurement, the internal solution contained (mmol/L) CsCl 130, NaCl 10, MgCl2, Cs2EGTA 5, HEPES 10, and ATP 4, pH 7.4. The external solution contained (mmol/L) NaCl 40, tetraethylammonium chloride 95, KCl 5.4, CaCl2 1, CoCl2 3, MgCl2 1, HEPES 10, and glucose 10, pH 7.4.

**Statistical Analysis**

Statistical analysis was performed by ANOVA. Where appropriate, simple comparisons were made by paired t test. Results are expressed as mean ± SD as an index of dispersion of values around the mean, except where otherwise indicated.

**Results**

We used the whole-cell patch-clamp technique13 to compare directly transmembrane Ca2+ currents in isolated cardiac myocytes from cardiomyopathic and age-matched normal hamsters. Two types of Ca2+ channel currents could be recorded from both myopathic and normal cells (Fig 1A). A test depolarization to −30 mV from a holding potential of −50 mV elicited a small inward current consistent with an L-type channel current that activated within 5 milliseconds and was well maintained during the depolarization. An additional transient component of inward current consistent with a T-type channel current was activated at the same test potential when the cell was held at −90 mV before depolarization. This current activated rapidly to peak and then decayed to zero within ~35 milliseconds. In cardiomyopathic cells, T-type current was a regular observation. However, in some normal cells (5 of 25), no T-type current was detectable. The two inward currents differed not only in their kinetics and dependence on the holding potential but also in their sensitivity to 40 μmol/L Ni2+ or 1 μmol/L nifedipine, as shown in Fig 1B. Fig 1C shows that the dihydropyridine Ca2+ channel agonist (+)PN 202-19914 selectively increased the maintained current component but failed to affect the transient current in cardiomyopathic cells. Similar results were found in normal cells (data not shown). The T-type current was not changed by 30 μmol/L tetrodotoxin (TTX) in either normal or myopathic cells.15 However, 30 μmol/L TTX completely blocked Na+ current in both myopathic and normal cells (data not shown). The overall concentration-effect curve for TTX on Na+ current was sigmoidal, with an EC50 of ~5 × 10−6 mol/L at a membrane potential of ~30 mV. No significant difference was observed in sensitivities to TTX of cardiomyocytes from normal and myopathic hamsters.

As shown in Fig 1A, in cardiomyopathic cells the peak magnitude of the T-type current was larger than that in normal myocytes, whereas the cells from normal and myopathic hamster hearts shown in this example had indistinguishable cell capacitance. We also compared the current density of T-type and L-type Ca2+ channels, since the mean cell capacitance was significantly greater in cardiomyopathic myocytes, presumably because of cell hypertrophy, compared with that in age-matched normal myocytes.11 With 20 mmol/L Ba2+ as the charge carrier, the current density of T-type Ca2+ channels in cardiomyopathic cells was significantly higher than that in normal cells (138 ± 1.8 and 5.8 ± 1.1 pA/pF, respectively), with a −90-mV holding potential and a −30-mV testing potential (n = 8, P < .01), as shown in Fig 2. For T-type Ca2+ channels in cardiomyopathic cells, the current-voltage relation was shifted in a hyperpolarizing direction when compared with the normal control cells. The peak of the current relation was at −30 mV for the normal cells and at −40 mV for the myopathic cells. These results indicate that there is a substantial increase in T-type Ca2+ channel current density in cardiac myocytes from cardiomyopathic hamsters.16

In contrast, as shown in Fig 2, when the membrane holding potential was −50 mV with a −10-mV testing potential, the maximal current was the same in myo-
pathic cells and normal cells (17.8±1.5 versus 18.6±2.1 pA/pF, respectively; n=8; P>.4). For L-type Ca\(^{2+}\) channels, when the holding potential was −50 mV, the current-voltage relation showed an apparent threshold at −40 mV, a maximum at −10 mV, and an apparent reversal potential at +40 mV in both cardiomyopathic myocytes and age-matched normal control cells. There was no statistically significant difference in each test potential–induced current change in myopathic and normal cells. These results demonstrate that there was no measurable difference in the properties of L-type dihydropyridine-sensitive channels in ventricular myocytes from cardiomyopathic hamsters compared with normal hamsters.

The kinetic properties of the two types of Ca\(^{2+}\) currents were compared further in cardiomyopathic and normal myocytes. In Fig 3A, superimposed tracings of T-type currents are shown, resulting from a protocol in which cells were depolarized to a command potential of −30 mV from various holding potentials.\(^{1,8}\) In Fig 3B, showing superimposed tracings of L-type currents, the currents were elicited at +20 mV from varying holding potentials. The peak amplitudes of both T- and L-type currents were normalized and plotted against the membrane potentials in Fig 3C and 3D. The degree of steady-state inactivation and activation of T- and L-type currents revealed a sigmoidal relation to voltage. The steady-state activation parameter (d\(_{1/2}\)) was obtained by normalizing the peak conductance (g\(_{max}\)) with the maximum available conductance (g\(_{max}\), the maximum value of g\(_{o}\)) as follows: d\(_{1/2}\) = g\(_{o}\)/g\(_{max}\) = (I\(_{L}\))/g\(_{o}\) (V\(_m\)−E\(_{rev}\)), where I\(_{L}\) is the peak Ca\(^{2+}\) current, V\(_m\) is membrane voltage, and E\(_{rev}\) is the reversal potential of the Ca\(^{2+}\) current.\(^{1,5}\) E\(_{rev}\) was assumed to be the zero-current potential in the current-voltage relation. The continuous curves in Fig 3C and 3D were calculated according to y = (1+exp(V\(_m\)−V\(_{0.5}\))/s)\(^{-1}\), where y is either f\(_i\) (inactivation parameter) or d\(_{1/2}\), V\(_{0.5}\) is the potential to give a half value, and s is the slope factor that defines the steepness of the curve. The inactivation and activation parameters

Fig 1. A, Two types of Ca\(^{2+}\) channels in cardiac myocytes from cardiomyopathic (CM) and normal (NH) hamsters. Whole-cell currents carried by Ba\(^{2+}\) (20 mmol/L) are shown in response to voltage-clamp steps. Two currents elicited from holding potentials of −50 and −90 mV are superimposed (upper tracings). The extra component of current activated from the more negative holding potential is shown as the difference between the two current tracings (lower tracings). The capacitance was 141 pF for the NH cell and 145 pF for the CM cell. B, Effects of Ni\(^{2+}\) and nifedipine on the T-type Ca\(^{2+}\) current in CM myocytes. Transient Ba\(^{2+}\) current elicited from a holding potential of −90 mV is shown. T-type currents before and during the administration of 10 μmol/L nifedipine were completely abolished in the T-type current. The same effect was found in NH cells (data not shown). Solutions and patch-clamp methods are the same as in panel A. C, Effects of the dihydropyridine Ca\(^{2+}\) channel agonist (+)PN 202-791 on two types of Ca\(^{2+}\) currents. The figure shows superposition of whole-cell Ba\(^{2+}\) currents elicited from two holding potentials. The same CM cell is shown as in panel A. Exposure to 1 μmol/L (+)PN 202-791 selectively increased the T-type current without effect on the T-type current. Lower tracing is T-type current isolated by subtracting the current of −50-mV holding potential from the current of −90-mV holding potential. The same effect was observed in NH cells.

Fig 2. Peak current-voltage relations of two types of Ca\(^{2+}\) channels in ventricular myocytes from normal (open symbols) and cardiomyopathic (filled symbols) hamsters. T-type Ca\(^{2+}\) current density is plotted for normal and myopathic cells (circles). The current was obtained at various test potentials from holding potentials of −90 and −50 mV. T-type current was identified by subtracting observed currents elicited from a holding potential of −50 mV from values observed using a holding potential of −90 mV. L-type Ca\(^{2+}\) current density is shown for normal and myopathic cells (squares). The current was obtained at various test potentials from a holding potential of −50 mV. Ca\(^{2+}\) currents were measured as peak inward currents in reference to zero current. Each point demonstrates mean±SEM of the current density (n=8 cells/4 hearts) of each Ca\(^{2+}\) channel type. Solutions and patch-clamp methods are the same as in Fig 1. Cell capacitance was measured from the capacitive current elicited by a small step depolarization.
were fitted well by the Boltzmann relation. Transient Ca\textsuperscript{2+} current in cardiomyopathic cells inactivated at more negative holding potentials and was half inactivated at \(-88.7\pm0.8\) mV. In comparison, normal cells were half inactivated at \(-76.8\pm0.6\) (n=6, \(P<.01\)). The slope factor of the steady-state inactivation curve was 9.0\pm0.8 in myopathic and 6.1\pm0.6 in normal cells. The steady-state inactivation curve of T-type current in myopathic myocytes was thus shifted \(+12\) mV negative to that in normal cells, whereas the activation curve of T-type current was shifted 8 mV negative (\(V_{0.5}=-53.1\pm0.4\) mV in myopathic cells and \(-43.2\pm0.7\) mV in normal cells). However, L-type Ca\textsuperscript{2+} currents revealed similar slope factors of steady-state inactivation and activation, as well as half-inactivated and activated potentials in cardiomyopathic cells (slope factors, 11.3\pm0.8 and 9.9\pm0.4, respectively; potentials, \(-25.6\pm0.9\) and \(-4.3\pm0.4\) mV, respectively) and normal cells (slope factors, 10.2\pm0.7 and 9.2\pm0.5, respectively; potentials, \(-25.3\pm0.8\) and \(-4.5\pm0.5\) mV, respectively) (n=6, \(P>.1\)). Thus, at a given holding potential, the cardiomyopathic myocytes had more Ca\textsuperscript{2+} channels available for activation. This difference was accounted for by T-type Ca\textsuperscript{2+} channels but not by L-type Ca\textsuperscript{2+} channels.\textsuperscript{20}
Fig. 4. Two types of Ca$^{2+}$ currents carried by 2 mmol/L Ca$^{2+}$ in a normal hamster (NH) cell compared with that in a cardiomyopathic (CM) cell. T-type current was isolated by subtraction as in Fig. 1. L-type current was elicited by pulse from -50 to -10 mV. Linear leak and capacitive currents were subtracted digitally. The capacitance was 135 pF for the NH cell and 143 pF for the CM cell.

In various tissues, it has been shown that Ba$^{2+}$ substitution for Ca$^{2+}$ in the extracellular solution changes the L-type current markedly without significantly affecting the T-type current.\textsuperscript{15,21} Fig. 4 shows selected original current tracings recorded from a normal and a cardiomyopathic cell in 2 mmol/L Ca$^{2+}$. With a physiological concentration of Ca$^{2+}$ in the bathing solution, peak T-type current was 75 pA (-30 mV) in the normal cell and 138 pA (-30 mV) in the myopathic cell. In contrast, peak L-type current was 169 pA (-10 mV) in the normal cell, which was not significantly different from 171 pA (-10 mV) observed in the myopathic cell. Fig. 5A shows peak current-voltage relations recorded from five normal and five myopathic cells after incubation with 5 mmol/L Ca$^{2+}$ and then after substituting extracellular Ca$^{2+}$ with equimolar Ba$^{2+}$. Similar to the observations made in other tissues,\textsuperscript{15,22} Ba$^{2+}$ substitution greatly augmented the L-type current and slowed its decay. The changes induced by different charge carriers were indistinguishable in normal and myopathic cells. As shown in Fig. 5B, in 2 mmol/L Ca$^{2+}$, the density of L-type current was 1.6±0.5 pA/pF in normal cells and 1.7±0.5 pA/pF in myopathic cells (n=8, P>.05). In 2 mmol/L Ba$^{2+}$, the peak current density increased to 3.8±0.7 pA/pF in normal cells and 3.9±0.9 pA/pF in myopathic cells (n=8). Changing the bath solution from 5 mmol/L Ca$^{2+}$ to 5 mmol/L Ba$^{2+}$, the peak L-type current density was increased from 4.1±1.1 to 8.2±1.2 pA/pF in normal cells (n=8, P<.01).

Fig. 5. A. Graph showing peak current-voltage (I-V) relation for L-type current in 5 mmol/L Ca$^{2+}$ (circles) and 5 mmol/L Ba$^{2+}$ (triangles) in the same normal (open symbols) or cardiomyopathic cells (filled symbols). Values in 5 mmol/L Ca$^{2+}$ were obtained 6 minutes before changing to 5 mmol/L Ba$^{2+}$. The current was obtained at various test potentials from a holding potential of -50 mV. Each point demonstrates mean±SEM of the current density of the L-type channel carried by 2 and 5 mmol/L Ca$^{2+}$ or Ba$^{2+}$ in normal (open bars) and cardiomyopathic (hatched bars) cells (n=8). Currents were measured after leak subtraction at the peak of the I-V relation in normal cells. C. Graph showing peak L-V relation for T-type current in 5 mmol/L Ca$^{2+}$ (triangles) and 5 mmol/L Ba$^{2+}$ (squares) in the same normal (open symbols) or cardiomyopathic cells (filled symbols). The current was obtained at various test potentials from holding potentials of -90 and -50 mV. T-type current was identified by subtracting observed currents elicited from a holding potential of -50 mV from values observed using a holding potential of -90 mV. Each point demonstrates mean±SEM of the current density (n=5). D, Mean±SEM of the current densities of the T-type channel carried by 2 and 5 mmol/L Ca$^{2+}$ or Ba$^{2+}$ in normal (open bars) and cardiomyopathic (hatched bars) cells (n=8). Currents were measured after leak subtraction at the peak of the I-V relation in normal cells.
and from 3.9±1.2 to 8.1±1.1 pA/pF in myopathic cells (n=8, P<0.01). Therefore, both the size and kinetics of the transient component are the same in both solutions (Fig 5C). As shown in Fig 5D, in normal cells, the peak current density of the T-type current in 2 mmol/L Ca"+ was 0.39±0.11 pA/pF, the same as that in 2 mmol/L Ba"+ (0.38±0.11 pA/pF, n=8, P>0.08). In cardiomyopathic cells, there was also no significant difference in the density of T-type current carried by 2 mmol/L Ca"+ (0.94±0.23 pA/pF) or 2 mmol/L Ba"+ (0.93±0.21 pA/pF, n=8, P>0.1). This suggests that Ca"+ and Ba"+ are about equally able to permeate through the T-type Ca"+ channel in both normal and cardiomyopathic cells. Since the kinetic differences between L- and T-type currents are more striking with Ba"+ as charge carrier than with Ca"+, using Ba"+ to enhance L-type Ca"+ current is also efficient for isolating T-type current from L-type current, facilitating comparison of channel properties between two groups of cells.22,23

The time course of decay of T-type currents during depolarization was studied by fitting the current change between the inward peak and current level 100 milliseconds after depolarization with a single-exponential function. As shown in Fig 4, the decay of T-type current during depolarization followed a single-exponential time course, and the time course was not markedly different whether Ca"+ or Ba"+ was the charge carrier.15,22 For Ba"+ -carried (20 mmol/L) T-type current induced by a −30-mV step from a holding voltage of −90 mV, the time course (τ) in myopathic cells was slightly increased (τ=14.8±4.7 milliseconds, n=15) compared with that in normal cells (τ=12.9±3.8 milliseconds, n=15) (Fig 1A). However, the area under the curve was still 78±21% more in cardiomyopathic cells than in normal cells. This implies that more Ca"+ could be transported through T-type Ca"+ channels in myopathic cells. The time course of L-type current carried by Ba"+ (induced by a 0-mV step from a holding voltage of −50 mV) was identical in normal and myopathic cells (Fig 4).

Discussion

Although this is the first report to focus on a comparison of both T- and L-type Ca"+ channel properties in cardiomyopathic and normal hamster cardiac myocytes, these experimental observations should be considered in the context of other studies of the properties of cardiac myocytes from the cardiomyopathic hamster. Other laboratories have reported that Ca"+ channel antagonists, when administered in vivo, preserve contractile function in myopathic hearts.24,25 Although these studies used drugs thought to act chiefly or entirely on L-type Ca"+ channels, a number of coronary and peripheral vascular as well as myocardial sites of action of Ca"+ channel antagonists could contribute to the salutary effect. Thus, we do not view the present observations as being at odds with a direct myocyte effect of verapamil.26

Recently Rossner27 reported a significant decrease in L-type Ca"+ channel density in cardiac myocytes from cardiomyopathic hamsters with end-stage heart failure (400 to 458 days old). The different finding in the present study may be due to the different age and disease stage at which the animals were studied. Klein and Houser28 reported that the slow inactivation component of L-type Ca"+ current was delayed and that the magnitude of peak L-type Ca"+ current was normal in early-stage but decreased in later-stage29 experimental pulmonary hypertension–induced hypertrophy of feline ventricular myocytes. In end-stage heart failure, the expression of several genes encoding Ca"+ -handling proteins in the heart has been found to be decreased, including the dihydropyridine receptor.30 It has been suggested that a reduction in L-type Ca"+ current may contribute to the decreased contractility observed in various cardiomyopathies accompanied by advanced congestive heart failure. However, such an abnormality in end-stage heart failure could not explain the pathogenesis of the cellular Ca"+ overload in the cardiomyopathic hamster at earlier stages. Thus, reduced L-type Ca"+ channel expression may represent only one of many consequences of myocyte dropout rather than a primary cause of the cardiomyopathy.

The L-type current density we observed in normal and myopathic cells is qualitatively lower than that reported by Rossner,27 possibly because of the different animal ages and different experimental conditions. The L-type current was recorded by Rossner with 135 mmol/L NaCl present in the extracellular solution without TTX. In the present study, tetraethylammonium aspartate was used instead of Na"+, and TTX was added to completely abolish the Na"+ current. The method of cell capacitance measurement was also different and might affect Ca"+ current quantification. We observed no significant time-dependent rundown/runup in L-type current from normal or myopathic cells during 30-minute recording intervals.

The physiological role of the T-type Ca"+ channel is not well delineated. Since the T-type Ca"+ channel current is largest in embryonic and neonatal cells, it has been thought of as a developmentally regulated channel. Xu and Best31 demonstrated that T-type current was significantly increased in atrial cells from cardiac hypertrophic rats with growth hormone-secreting tumors. Nuss and Houser32 recently reported that there is an increase in T-type channel density in ventricular myocytes from cats with experimental pressure overload–induced left ventricular hypertrophy. Hence, there is a suggestion that higher levels of T-type Ca"+ current might be associated with cell growth and therefore with cardiac hypertrophy.

The shift observed in T-type Ca"+ channel voltage dependencies of inactivation and activation might partially counteract the effect of an increase in T-type Ca"+ channel density at the normal resting potential. However, as we have shown in Figs 2 and 4, even with 42% channel inactivation, the density of the T-type Ca"+ channel current in cardiomyopathic cells is still 2.3-fold higher than in normal cells. At a −80-mV holding potential, the T-type Ca"+ current was still 1.8-fold higher in myopathic than in normal cells. This significant increase in T-type current density could not be completely abolished by the observed voltage shift in inactivation. Interestingly, this shift in voltage dependencies could move the voltage range for the T-type window current toward the resting potential.33 This abnormality might promote spontaneous beating or arrhythmias in myopathic tissue.34

The experimental data presented here permit several conclusions regarding Ca"+ homeostasis in the Bio 14.6
cardiomyopathic hamster heart at the stage studied. The L-type Ca\textsuperscript{2+} channels show no demonstrable abnormality as judged by whole-cell clamp studies, in agreement with our finding of no appreciable difference in the numbers of dihydropyridine binding sites in cells from the same source.\textsuperscript{20} The lack of change in affinity for a dihydropyridine ligand is also consistent with the absence of any major change in the structure of the channel, at least in the dihydropyridine binding domain. Although no significant difference in Na\textsuperscript{+} current density was observed between myopathic and age-matched normal cells, we cannot exclude the possibility that there is abnormal Na\textsuperscript{+} channel expression, since the present study was done with only 40 mmol/L Na\textsuperscript{+} in the extracellular solution. Measurement of Na\textsuperscript{+} current with more physiological [Na\textsuperscript{+}] present is needed to compare the Na\textsuperscript{+} current density and kinetics in this experimental model.

Our results indicate that there is considerably increased T-type Ca\textsuperscript{2+} channel current density in cardiac myocytes from cardiomyopathic hamsters and that these channels show abnormal activation and inactivation kinetics. This change in T-type Ca\textsuperscript{2+} channel properties may be a clue to the pathogenetic process, but its role in Ca\textsuperscript{2+} overload is not clear. The abnormal T-type Ca\textsuperscript{2+} channel properties in this genetically determined cardiomyopathy suggest that this alteration may contribute to the pathogenesis of Ca\textsuperscript{2+} overload as a consequence of enhanced transsarcolemmal Ca\textsuperscript{2+} influx through this pathway. The increase of the T-type Ca\textsuperscript{2+} channel current might also contribute to the abnormalities in electrophysiological properties and arrhythmogenic potential in this form of cardiomyopathy.

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L Sen and T W Smith

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