Rapid Communication

T-Type Ca\(^{2+}\) Current Is Expressed in Hypertrophied Adult Feline Left Ventricular Myocytes

H. Bradley Nuss, Steven R. Houser

Macroscopic T-type Ca\(^{2+}\) currents, which are often observed in fetal and neonatal cardiac muscle cells, were not found in normal (0 of 17) adult feline ventricular myocytes. However, they were present in most (15 of 21) myocytes isolated from adult feline left ventricles with long-standing pressure-overload–induced hypertrophy. This is the first study to provide evidence in a large mammal, such as the cat, that T-type Ca\(^{2+}\) channels may be reexpressed in adults in association with hypertrophy resulting from slow progressive pressure overload. Importantly, this expression was stable for the duration of the hypertrophy process and was not associated with abrupt pressure overload. T-type Ca\(^{2+}\) currents were separated from L-type Ca\(^{2+}\) currents by exploiting the differences in their voltage dependence of steady-state inactivation. Depolarizations from -80 mV revealed a rapidly activating inward current that peaked in magnitude at -30 mV (-1.8±0.9 [mean±SD] pA/pF) and fully inactivated within 100 milliseconds in 15 of 21 hypertrophied myocytes studied. Further depolarizations activated progressively less T-type Ca\(^{2+}\) current, so that at +10 mV the L-type Ca\(^{2+}\) current predominated. In the hypertrophied myocytes that demonstrated both T-type and L-type Ca\(^{2+}\) currents, two distinct peaks occurred in their current-voltage relations. T-type Ca\(^{2+}\) currents were not evident in any of the 17 normal adult feline left ventricular myocytes studied. The purpose of T-type Ca\(^{2+}\) currents in hypertrophy is unclear. However, their presence may make hypertrophied myocardium more prone to spontaneous action potentials and increase the likelihood for arrhythmias in partially depolarized hypertrophied myocardium. (Circ Res. 1993;73:777-782.)

KEY WORDS • T-type Ca\(^{2+}\) currents • cardiac hypertrophy • ventricular myocytes • patch clamp

Hemodynamic overloading, such as pressure overload, is one factor known to stimulate the adult heart to enter an active growth phase.\(^1\) Slow progressive pressure overload produces severe hypertrophy of the myocytes that make up the overloaded ventricle.\(^2-4\) Associated with this hypertrophic growth is prolongation of the myocyte action potential duration\(^6\) and a propensity for arrhythmias.\(^7\) The membrane basis for the electrophysiological alterations of the hypertrophied myocyte has been examined. These studies have shown that, in general, small but significant alterations in L-type Ca\(^{2+}\) current (I\(_{CaL}\)),\(^5,8\) delayed rectifier K\(^+\) current,\(^6,9\) and inward rectifier K\(^+\) current\(^6\) contribute to the action potential prolongation of the hypertrophied myocyte. The putative role of the T-type Ca\(^{2+}\) current (I\(_{CaT}\)) in the electrical or mechanical changes during hypertrophy has not, to our knowledge, been studied to date. One possibility is that alterations in T-type Ca\(^{2+}\) channel density are involved in hypertrophy-related arrhythmias.

The objective of this study was to determine if the expression of I\(_{CaT}\) is altered with hypertrophy. We have not previously observed I\(_{CaT}\) in normal adult feline ventricular myocytes.\(^10\) However, there is some evidence that T-type Ca\(^{2+}\) channels are developmentally regulated and that expression varies with cardiac cell type.\(^11-13\) In support of this idea, the largest I\(_{CaT}\) density has been recorded in embryonic chick ventricular cells.\(^14\) In addition, in mouse\(^15\) and rat skeletal muscle, I\(_{CaT}\) decreases in a progressive manner during postnatal development. Since hypertrophy recapitulates the fetal phenotype,\(^17,18\) it seemed possible that I\(_{CaT}\) might be altered in the hypertrophied heart. In the present study, we confirmed that I\(_{CaT}\) was not present in normal adult feline ventricular myocytes and observed that it was present in a large percentage of hypertrophied myocytes.

Materials and Methods

Model of Left Ventricular Hypertrophy

The chronic feline model of left ventricular hypertrophy used in this study was recently described in detail\(^2\) and was modified from a model of right ventricular hypertrophy described by Cooper et al.\(^19\) A band of fixed diameter was placed around the aorta of immature animals (1 kg) such that minimal constriction of the aorta occurred at the time of surgery. A slow progressive pressure overload developed on the left ventricle during the growth of the animals into adulthood.\(^2\) This long-standing pressure overload resulted in severe cardiac hypertrophy, and myocytes isolated from these hearts exhibited alterations in both electrophysiological and contractile properties.\(^2,8\)

Received March 26, 1993; accepted June 30, 1993.
From the Department of Physiology, Temple University School of Medicine, Philadelphia, Pa.
Correspondence to Steven R. Houser, PhD, Temple University School of Medicine, Department of Physiology, 3420 N. Broad Street, Philadelphia, PA 19140.
Isolation of Ventricular Myocytes

Myocytes were disaggregated from feline hearts using cell isolation techniques developed in this laboratory with slight modifications. On the day of study, the animal was given an intramuscular injection of ketamine (50 mg/kg) and acepromazine (0.5 mg/kg). The heart was then rapidly excised and rinsed in saline until the chambers were clear of blood. After cannulation of the aorta, the coronary were perfused with nominally Ca²⁺-free modified Krebs-Henseleit buffer (KHB) until the coronary circulation was cleared of blood cells. Perfusion was continued with recirculating KHB containing collagenase (180 U/mL, type II, Worthington Biochemical Corp, Freehold, NJ) and gassed with 95% O₂–5% CO₂ to maintain pH at 7.4. The flow rate was adjusted to achieve an initial perfusion pressure of 80 to 100 mm Hg. After the perfusion pressure dropped and the heart tissue became flaccid, the left ventricle was dissected free from the rest of the heart. This tissue was minced in the remaining KHB solution containing collagenase and gently shaken for 5 minutes in a shaking water bath. The cells were filtered through 300-µm meshing, rinsed with KHB, centrifuged, and resuspended in KHB containing 1% serum albumin and 1 mmol/L CaCl₂. The cells were kept at room temperature (24°C) and gassed with 95% O₂–5% CO₂. All solutions used in the isolation procedure were maintained at 37°C. Only myocytes from the left ventricles of normal and aortic-banded cats were used in these experiments. All experiments were performed within 16 hours of isolation.

Solutions

A drop of cell-containing solution was placed in the experimental chamber. After the myocytes had settled to the bottom, perfusion was begun with a modified Tyrode’s solution composed of (mmol/L) NaCl, 150; KCl, 5.4; MgCl₂, 1.2; dextrose, 10; pyruvate, 2.5; HEPES, 5; and CaCl₂, 1 (pH 7.4). The pipette filling solution was composed of (mmol/L) CsCl, 125; tetra-ethylammonium chloride (TEA-Cl), 20; EGTA, 10; HEPES, 10; and K₂-ATP, 5 (pH 7.3).

Upon the attainment of gigahm seals, suction was used to disturb the patch of membrane enclosed by the pipette tip. Dialysis of the pipette filling solution occurred in 3 to 5 minutes using 2- to 3-MΩ pipettes as evidenced by depolarization of membrane potential from ~70 mV to less than ~40 mV. The cells were then superfused with the 0 Na⁺ recording solution without developing contracture. Depolarizations from ~80 to ~40 mV were used to monitor the exchange of solutions. After the disappearance of the Na⁺ current, an additional 3 minutes of continuous perfusion with the recording solution ensured that the removal of Na⁺ from the bathing solution was complete. The recording solution was composed of (mmol/L) choline chloride, 140; CsCl, 5; dextrose, 5.5; HEPES, 5; MgCl₂, 0.5; 4-aminopyridine, 2; and CaCl₂, 5 (pH 7.4). All experiments were conducted at 35°C.

Whole-Cell Voltage Clamp

Whole-cell membrane currents were recorded by the patch-clamp method described by Hamill et al. Pipettes were fabricated from filament-containing glass capillary tubes (1.5 mm outer diameter, World Precision Instruments, Sarasota, Fla) on a microelectrode puller (model P-87, Sutter Instruments Co, Novato, Calif) and then fire-polished. Pipettes had tip resistances of 2 to 3 MΩ when filled with the internal solution. The bath potential was recorded with a 3 mol/L KCl agar–Ag/AgCl reference electrode. Voltage clamp was achieved using the discontinuous switch clamp technique (Axoclamp-2 amplifier, Axon Instruments, Burlingame, Calif). Careful adjustment of capacity neutralization, steady-state gain, and phase shift produced capacity transients with time constants of less than 1 millisecond. The maximum peak voltage error produced when recording a peak current of 3 nA in a myocyte with an input capacitance of 250 pF was calculated to be in the range of 1 to 2 mV at a switching frequency of 10 to 15 kHz.

The voltage-clamp protocols were controlled using pCLAMP software (version 5.5.1, Axon Instruments) on an IMB-AT computer. Membrane currents were digitized on-line with 12-bit resolution using a Labmaster interface analog to digital converter (Axon Instruments) and stored on hard disk for off-line analysis.

Myocyte input capacitance was calculated from data obtained while in the current-clamp mode immediately after obtaining access to the cell. Briefly, the slope conductance (ratio of current to voltage) was calculated from a small hyperpolarizing current step and the resultant voltage change. The membrane time constant was calculated from the decay of voltage at the end of the current pulse. The input capacitance was given by the product of the slope conductance and the membrane time constant.

Data Analysis and Statistics

Peak current measurements and curve-fitting were accomplished using pCLAMP software (version 5.5.1, Axon Instruments). All measurements are reported as mean ± SD. Student’s t test was used to determine if measurements in hypertrophied myocytes were significantly different from measurements in normal myocytes.

Results

Hemodynamics and Cell Properties

The general characteristics of the animals used in this study have been reported previously. In brief, there were large increases, more than 70 mm Hg, in the pressure gradient across the constricting band as the animals grew. In addition, the ratio of heart weight to body weight increased by more than 100%, documenting severe hypertrophy in response to this pressure overload. Myocytes isolated from these hearts had prolonged action potential and contractile durations characteristic of hypertrophied myocardium. Myocytes from these same hearts were used in the experiments described below.

Separation of Icao and ICa,T

The voltage-clamp protocols were designed to identify with certainty macroscopic Icao in normal and hypertrophied feline ventricular myocytes. Approximately 40 mV separates the voltage dependence of steady-state inactivation of Icao and ICa,T. Preliminary experiments confirmed that conditioning pulses (7
seconds) to −80 mV removed inactivation from functional T-type Ca\(^{2+}\) channels when present. Conditioning pulses to −40 mV inactivated T-type Ca\(^{2+}\) channels, when present, without affecting L-type Ca\(^{2+}\) channel availability. Removal of Na\(^{+}\) from the bath and internal solutions eliminated both tetrodotoxin-sensitive and tetrodotoxin-resistant Na\(^{+}\) currents in feline ventricular myocytes.27 Outward K\(^{+}\) currents were minimized by the substitution of Cs\(^{+}\) for K\(^{+}\) in combination with internal TEA-Cl (20 mmol/L) and external 4-aminopyridine (2 mmol/L). Depolarizations from −80 mV could elicit both \(I_{Ca,T}\) and \(I_{Ca,L}\), whereas depolarizations from −40 mV would elicit only \(I_{Ca,L}\). The difference in the current recordings obtained from the two conditioning potentials represents the \(I_{Ca,T}\) elicited at that test potential.

Preliminary experiments indicated that small amounts of rundown of \(I_{Ca,L}\) or small changes in the amount of leak current could hamper the identification of \(I_{Ca,T}\) in the difference currents. In addition, a depolarized conditioning potential had to be selected so that \(I_{Ca,L}\) was not partially inactivated.26 To avoid these problems, the voltage was stepped to a given test potential first from a holding potential of −80 mV and 7 seconds later from −40 mV. The −40-mV conditioning potential, used to inactivate \(I_{Ca,T}\), did not inactivate any \(I_{Ca,L}\) because the Ca\(^{2+}\) currents elicited after the −40- and −80-mV conditioning pulses were identical at 0 mV and more positive test potentials (Figs 1 and 3).

\(I_{Ca,T}\) in Hypertrophied Myocytes

\(I_{Ca,T}\) was not evident in any of the current recordings obtained from the 17 normal myocytes studied from eight different cats (Fig 1). The Ca\(^{2+}\) currents elicited after the −80-mV conditioning pulse were measurably identical in peak magnitude to \(I_{Ca,L}\) elicited from −40 mV at every test potential (Fig 2). On the basis of this negative finding and previous unpublished negative observations from this laboratory,10 we suggest that normal adult feline ventricles either lack or have a very low density of functional T-type Ca\(^{2+}\) channels.

Significant \(I_{Ca,T}\) were recorded in 15 of 21 hypertrophied ventricular myocytes studied (Fig 3). The remaining 6 hypertrophied myocytes did not have an obvious \(I_{Ca,T}\). Hypertrophied myocytes were studied from nine different aortc-banded cats.

In the hypertrophied myocytes, \(I_{Ca,T}\) first became evident on depolarization to −45 mV, peaked in magnitude at −30 mV, grew smaller with further depolarizations, and became undetectable by 0 mV (Fig 4). Peak \(I_{Ca,T}\) measured in the hypertrophied myocytes measured −0.47±0.25 nA (n=15) with 5 mmol/L external Ca\(^{2+}\). \(I_{Ca,T}\) magnitudes were measured as the difference between the peak current and the steady-state current at 500 milliseconds. The corresponding \(I_{Ca,T}\) density was −1.8±0.9 pA/pF (n=14) once normalized by myocyte input capacitance. \(I_{Ca,T}\) inactivation at −30 mV was best fit by a single exponential decay with an inactivation time constant (\(\tau\)) of 9.3±1.8 milliseconds (n=14). This value is similar to those defined by others in previous studies.11,25,28 When myocytes were exposed to Cd\(^{2+}\) (50 to 200 μmol/L CdCl\(_2\)), \(I_{Ca,L}\) was eliminated, but a significant portion (approximately 50%) of \(I_{Ca,T}\) remained (data not shown). The remaining \(I_{Ca,T}\) could be eliminated by either Ni\(^{2+}\) (1 mmol/L NiCl\(_2\)) or removal of extracellular Ca\(^{2+}\). These data show that the current observed in hypertrophied myocytes has properties similar to \(I_{Ca,T}\) studied previously.11,24,29

\(I_{Ca,L}\) in Hypertrophied Myocytes

With 5 mmol/L Ca\(^{2+}\) as the charge carrier, peak \(I_{Ca,L}\), elicited from −40 to +10 mV, in hypertrophied left ventricular myocytes (−2.8±0.8 nA, n=21) was not different from normal myocytes (−2.8±0.9 nA, n=17). However, after correction for individual myocyte input capacitance, peak \(I_{Ca,L}\) density in hypertrophied myocytes (−11.3±1.9 pA/pF, n=19) was significantly (\(P<.01\)) reduced to 77% of normal (−14.6±3.3 pA/pF, n=16) at +10 mV. These data are summarized in Fig 5. Inactivation of \(I_{Ca,L}\) at +10 mV was best described as a biexponential decay process. The time course of both the fast and slow decay processes were slower for hypertrophied (\(\tau_{fast}\), 14.4±2.7 milliseconds; \(\tau_{slow}\), 5.3±1.1 milliseconds; n=21) than normal (\(\tau_{fast}\), 10.4±1.7 milliseconds; \(\tau_{slow}\), 78.1±9.9 milliseconds; n=17) myocytes. Only the fast component was slowed significantly (\(P<.01\)). The magnitude of the slow and maintained components of \(I_{Ca,T}\) were reduced significantly in hypertrophied myocytes (see the Table). These results are similar to those we have observed previous-
ly. They show that peak $I_{\text{Ca,L}}$ density is reduced in severely hypertrophied myocytes. Total Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels may not be significantly altered because $I_{\text{Ca,L}}$ inactivation is slowed.

Thus, $I_{\text{Ca,T}}$ has been associ-
Inactivation Parameters of L-Type Ca\(^{2+}\) Current

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\tau_{\text{fast}})</td>
<td>10.4±1.7 ms</td>
</tr>
<tr>
<td>(\tau_{\text{slow}})</td>
<td>78.1±9.9 ms</td>
</tr>
<tr>
<td>(\text{Amp}_{\text{fast}})</td>
<td>14.4±2.7 nA</td>
</tr>
<tr>
<td>(\text{Amp}_{\text{slow}})</td>
<td>80.5±14.1 nA</td>
</tr>
<tr>
<td>SS</td>
<td>1.7±0.7 nA</td>
</tr>
</tbody>
</table>

\(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\) indicate fast and slow time constants of inactivation, respectively; \(\text{Amp}_{\text{fast}}\) and \(\text{Amp}_{\text{slow}}\), fast and slow amplitudes, respectively; SS, maintained (steady-state) component; NM, normal myocytes; and HM, hypertrophied myocytes. Values are means±SD.

The inactivation of the L-type Ca\(^{2+}\) current in 17 NM and 21 HM was fit as a bieponential decaying process.

\*P<.01 vs NM.

ated with active growth phases, suggesting that it is developmentally regulated. The present results suggest that the cell-signaling process that produces hypertrophy also results in a reduced density of one type of Ca\(^{2+}\) channel (L-type) and an increased density of another type (T-type), implying differential regulation.

**Gene Expression in Hypertrophy**

A number of previous studies support the idea that an increase in hemodynamic loading in adults signals a general reexpression of fetal phenotypes (see References 17 and 31). Pressure or volume overloading of the adult rat heart induces \(\beta\)-myosin heavy chain messenger RNA and \(\beta\)-myosin heavy chain proteins, which comprise \(V_1\) myosin.\(^{3,34}\) The \(V_1\) myosin isoforms predominantly in embryonic ventricle,\(^{35}\) is replaced by \(V_1\) myosin in the young adult, but is reexpressed in hypertrophied adult ventricles.\(^{3,33}\) The idea that hypertrophy induces genes encoding fetal proteins has recently been reviewed.\(^{17,31}\) The fact that \(I_{\text{Ca,T}}\) predominates over \(I_{\text{Ca,L}}\) in embryonic ventricles,\(^{14}\) is small or absent in adult ventricular myocytes, and then reemerges in hypertrophied adult feline ventricles is consistent with the idea that the T-type Ca\(^{2+}\) channel gene(s) is developmentally regulated.

**\(I_{\text{Ca,T}}\)-Mediated Ca\(^{2+}\) Influx in Hypertrophy**

The mean \(I_{\text{Ca,T}}\) density recorded in hypertrophied feline ventricular myocytes (\(-1.8±0.9\) pA/pF) is comparable to that found in rabbit sinoatrial node cells (\(-2.1\) pA/pF)\(^{29}\) and canine Purkinje cells (\(-1.7\) and \(-2.9\) pA/pF)\(^{11,25}\). Smaller \(I_{\text{Ca,T}}\) densities have been recorded in rat atrial (\(-0.4\) pA/pF)\(^{30}\) and guinea pig ventricular (\(-0.6\) pA/pF)\(^{26}\) myocytes. Larger \(I_{\text{Ca,T}}\) densities have been recorded only in chick embryonic (\(-4.2\) pA/pF)\(^{34}\) and cultured neonatal rat (\(-3.0\) pA/pF)\(^{28}\) ventricular myocytes.

Our finding of \(I_{\text{Ca,T}}\) in hypertrophied ventricular myocytes but not in normal adult ventricular myocytes (at least with the approach we used) suggests that Ca\(^{2+}\) influx via T-type Ca\(^{2+}\) channels might be associated with cell growth. Xu and Bent\(^{30}\) found that growth hormone-secreting tumors, which result in volume overload and cardiac hypertrophy, increase \(I_{\text{Ca,T}}\) in rat atrial myocytes. Furukawa et al\(^{30}\) found that the vasoconstrictive peptide endothelin-1, which induces hypertrophy and increased DNA and protein synthesis,\(^{34}\) increases \(I_{\text{Ca,T}}\) in cultured neonatal rat ventricular myocytes. The regulation of \(I_{\text{Ca,T}}\) expression and its precise link to the growth that occurs in cardiac hypertrophy cannot be fully studied at present because the gene(s) encoding this membrane protein has not, to our knowledge, yet been cloned.

**Expression of T-Type Ca\(^{2+}\) Channels in Hypertrophy**

We have found evidence that T-type Ca\(^{2+}\) channels are expressed during hypertrophy in the adult feline ventricle, where there is normally no measurable \(I_{\text{Ca,T}}\), and that the expression of these channels is stable throughout long-standing hypertrophy. The expression of T-type Ca\(^{2+}\) channels in the adult feline ventricle may be intimately associated with its reentry into an active growth phase during pressure-overload hypertrophy. In addition to being involved in growth and development, \(I_{\text{Ca,T}}\) could also influence basic electrophysiological characteristics of the hypertrophied ventricle, thereby making hypertrophied hearts more prone to arrhythmias.

**Acknowledgments**

This study was supported by National Institutes of Health Grant RO1 HL-33921 to Dr Houser.

**References**

2. Bailey BA, Houser SR. Calcium transients in fetal left ventricular myocytes with hypertrophy induced by slow progressive pressure overload. *J Mol Cell Cardiol*. 1992;24:365-373.
T-type Ca2+ current is expressed in hypertrophied adult feline left ventricular myocytes.
H B Nuss and S R Houser

Circ Res. 1993;73:777-782
doi: 10.1161/01.RES.73.4.777

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/73/4/777