Calcium Current Is Increased in Isolated Adult Myocytes From Hypertrophied Rat Myocardium

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To study the effects of myocardial hypertrophy resulting from chronic pressure overload on excitation-contraction coupling, the cardiac transmembrane L-type calcium current (I_Ca) was investigated in the Goldblatt renovascular hypertensive (HBP) rat. I_Ca was measured in single myocytes enzymatically isolated from control (CTRL) and HBP rat hearts using the whole-cell, patch-clamp method. The peak I_Ca and I_Ca density (obtained by normalizing I_Ca to the average cell capacitative surface area) were larger in HBP cells (n=15) than in CTRL cells (n=10) at membrane potentials of -20 to 50 mV (p<0.01). The maximal peak I_Ca increased from 0.9±0.5 nA (mean±SD) in CTRL cells to 2.8±1.0 nA in HBP cells (p<0.001). The corresponding I_Ca density increased from 5.3±2.7 to 16.2±6.0 μA/cm² (p<0.001). There was no shift in the current-voltage relation between CTRL and HBP cells. The time course of decay of HBP I_Ca in response to clamp steps to the plateau range of the action potential (membrane potential, V_m= -10 to 30 mV) was delayed when compared with that of CTRL I_Ca. The inactivation time constants (biexponential) for the maximal I_Ca were 6.9±1.9 and 36.0±9.3 msec for CTRL cells and 6.7±1.4 and 49.5±12.9 msec for HBP cells (p<0.05 for the slower component of the maximal I_Ca). There was no difference in the steady-state inactivation of I_Ca (f_inact) for the CTRL and HBP cells. From the maximal peak I_Ca, cytoplasmic free Ca²⁺ was estimated to reach a pCa of 6.95±0.07 for CTRL cells and 6.64±0.13 for HBP cells. It is concluded that I_Ca is increased with myocardial hypertrophy. The lengthening of the action potential in hypertrophied rat myocardium is due to an increase in peak current density and to the slower inactivation of the maximal I_Ca. The increased transmembrane flux of Ca²⁺ via I_Ca in HBP cells is inadequate to achieve a myoplasmic free Ca²⁺ level sufficient for direct partial activation of the contractile myofilaments. However, in the scheme of the calcium-triggered calcium release hypothesis such an increase could provide an increased amount of activator calcium and/or serve to amplify the release of Ca²⁺ from sarcoplasmic reticulum, thereby contributing to preserved peak developed tension in hypertrophied rat myocardium. (Circulation Research 1989;64:753–763)

In recent years, a large volume of information concerning contractile, electrophysiological, biochemical, and ultrastructural changes in myocardial hypertrophy in the two-kidney, one-clip Goldblatt renovascular hypertensive (HBP) rat has been accumulated. Developed isometric tension of papillary muscle from nonfailing hypertrophied rat left ventricle is increased or remains unchanged, whereas the time to peak relaxation and time to half relaxation are prolonged, and the velocity of shortening is depressed. Action potential duration is increased, and the effective membrane capacity is decreased. It is well established that the transmembrane action potential plays a key role in excitation-contraction coupling in heart muscle. There is a direct relation between the duration of the plateau phase of the action potential, the amount of transsarcolemmal flux of activator calcium, and developed tension. Furthermore, the role of the transmembrane L-type calcium current (I_Ca) in the maintenance of action potential duration and as the source of activator calcium in triggering the release of calcium from the sarcoplasmic reticulum (SR) has been well recognized.

There has been only one previous study on the effects of myocardial hypertrophy on transmem-
brane ionic currents. Analyses and characterization of the ionic channels has been hampered by the limitations of the sucrose-gap, voltage-clamp technique used in the investigation. Most of these limitations have been minimized by recent advances in single-cell, voltage-clamp techniques. However, there is no voltage-clamp study on the effects of myocardial hypertrophy on ionic currents in which isolated single myocytes from hypertrophied hearts were used. Because of the important role of in initiating excitation-contraction coupling in the cardiac muscle, using the whole-cell, voltage-clamp method I studied the effects of chronic pressure overload and cardiac hypertrophy on via isolated from hypertrophied hearts of HBP rats. was shown to be significantly increased in hypertrophied rat myocardium. There was slowing in the inactivation kinetics of . It was estimated that the increased transmembrane flux of via in hypertrophied rat myocardium was inadequate to achieve a myoplasmic free level sufficient for direct partial activation of the contractile myofilaments.

Materials and Methods

Hypertensive Model

All experimental procedures were approved by the San Francisco Veterans Administration Medical Center's Committee on Animal Research. Male rats (Bantin and Kingman, Fremont, California) weighing 150-200 g were made hypertensive by a procedure previously described. A silver clip (0.22-mm aperture) was placed around the left renal artery of the experimental rats under anesthesia with methoxyflurane. Age-matched rats were used as controls because previous work showed that the sham-operated rats were not different from unoperated control rats. The animals were maintained on water and standard rat chow. Systolic blood pressure was measured by the tail-cuff method. Hypertension was defined as a systolic blood pressure greater than or equal to 150 mm Hg. Experiments were performed 8 to 12 weeks after the clipped rats developed hypertension.

Cell Isolation

Single ventricular cells were isolated by a modification of the procedure used by Silver et al. The animal was anesthetized with methoxyflurane, and the heart was rapidly excised and attached to a Langendorff perfusion apparatus. The heart was then retrogradely perfused for 2-3 minutes with nominally calcium-free Krebs-Henseleit solution equilibrated with 95% O\textsubscript{2}-5% CO\textsubscript{2} at 35°C. The Krebs-Henseleit solution contained (mM) NaCl 137, KCl 5.4, MgCl\textsubscript{2} 1.0, CaCl\textsubscript{2} 1.8, HEPES 10, and glucose 10 at pH 7.4 (titrated with HCl). The internal pipette solution contained (mM) CsCl 140, MgCl\textsubscript{2} 2, HEPES 10, CaCl\textsubscript{2} 1, EGTA 11, K\textsubscript{2}ATP 5 at pH 7.2 (titrated with CsOH). The fast inward sodium current was inactivated by holding the membrane at -40 mV. Potassium currents were eliminated by inclusion of Cs\textsuperscript{+} in the pipette solution and by removal of K\textsuperscript{+} in the external solution after 20 minutes. The ventricles were separated from the atria and cut into small pieces and further digested with the recirculating enzyme solution plus 1% bovine serum albumin for 10 minutes in an atmosphere of 95% O\textsubscript{2}-5% CO\textsubscript{2} at 35°C. Cells were filtered, washed with increasingly higher concentrations (from 0 to 1 mM CaCl\textsubscript{2}) of calcium containing Krebs-Henseleit solution, and, finally, suspended in minimum essential medium containing 3% horse serum and stored in a cell incubator for 30 minutes to 1 hour before experiments.

Voltage-Clamp Experiments

Voltage-clamp experiments were performed in the whole-cell configuration with the single-pipette, patch-clamp method by use of either a Dagan 8900 patch/whole-cell clamp system (Dagan, Minneapolis, Minnesota) or an Axopatch-1B patch-clamp system (Axon Instruments, Inc, Burlingame, California). Pipettes were fabricated from 1.8-mm o.d. Microstar glass tubing (Radnoti Glass, Monrovia, California) by use of a two-stage pull with a Kopf 700D vertical pipette puller or with a micropipette-based patch pipette puller (PC-84, Sutter Instruments, San Rafael, California). The pipettes had an internal tip diameter of 2-2.5 μm after slight fire polishing and a resistance of 0.8-1.7 MΩ after backfilling with the experimental internal solution. Seals of 1-4 GΩ between the pipette tip and the cell membrane were achieved in an external solution containing (mM) NaCl 137, KCl 5.4, MgCl\textsubscript{2} 1.0, CaCl\textsubscript{2} 1.8, HEPES 10, and glucose 10 at pH 7.4 (titrated with HCl). The internal pipette solution contained (mM) CsCl 140, MgCl\textsubscript{2} 2, HEPES 10, CaCl\textsubscript{2} 1, EGTA 11, K\textsubscript{2}ATP 5 at pH 7.2 (titrated with CsOH). The fast inward sodium current was inactivated by holding the membrane at -40 mV. Potassium currents were eliminated by inclusion of Cs\textsuperscript{+} in the pipette solution and by removal of K\textsuperscript{+} in the external solution after the whole-cell configuration was achieved. All experiments were performed at an ambient temperature of 22-25°C.

Data Acquisition and Analysis

During the experiments, voltage and current data were displayed on a storage oscilloscope (5111A, Tektronix, Beaverton, Oregon). Analog waveforms for voltage-clamp command pulses and data acquisition and analysis were accomplished with a set of commercially available software programs (pClamp, Axon Instruments, Burlingame, California) using an 80 KHz Tecmar Labmaster analog-digital conversion board (Scientific Solutions, Tecmar Inc, Cleveland, Ohio) and an IBM-AT personal computer. Current traces elicited by voltage steps were filtered at a corner frequency of 5 KHz by an eight-pole
low-pass Bessel Filter, digitized at 10–500-μsec intervals and stored in the microcomputer for later analysis.

Current time constants were determined by least-squares fitting of a two-exponential function to the current trace. The data for the steady-state inactivation of \( I_{\text{Ca}} \) were fitted to a Boltzmann distribution of the form:

\[
f_n = \frac{1}{1 + \exp[(V + V_{0.5})/b]}
\]

where \( f_n \) is the steady-state inactivation parameter, \( V \) is the membrane voltage potential, \( V_{0.5} \) is the membrane voltage potential at which \( f_n \) equals 0.5 (i.e., when half of the calcium channels are available for activation), and \( b \) is a constant. The curve-fitting algorithm used for the steady-state inactivation curve was based on the Gauss-Newton method for the least-squares fit to a nonlinear function. The fitting procedure was performed by a commercially available software program (ASYST, Macmillan Software Company, New York, New York). It was continued until the size of the parameter adjustments at each step became smaller than a tolerance factor of 0.001.

Statistical significance was determined by Student’s \( t \) test for unpaired data unless stated otherwise. Data are presented as mean±SD.

Cell Capacity and Series Resistance

The cell capacity current and the total series resistance (electrode and internal resistance) are the major sources of error in single-electrode, whole-cell voltage-clamp experiments. If the capacity transient is still substantial at the time the calcium current peaks, an “instantaneous” voltage step change cannot be achieved, and the decay of the transient will prevent accurate measurement of the current. In the presence of a large uncompensated series resistance, the relation of the calcium current to the applied clamp potential will be skewed.\(^{28}\) To assess the limitations of the single-pipette, voltage-clamp method, cell capacitance and series resistance were estimated from the capacitative transient produced by a 10-mV hyperpolarizing voltage-clamp step from the resting membrane potential (Figure 1). The cell capacity (\( C_m \)) was defined as the ratio of the area under the current transient (total charge), photographed from the oscilloscope with Polaroid film, to the magnitude of the hyperpolarizing step (–10 mV). Before electronic compensation for \( C_m \) and series resistance, the mean \( C_m \) was 172±49 pF for cells isolated from the control (CTRL) rat hearts (\( n = 34 \)) and 169±49 pF for cells of comparable dimensions (\( p = 0.83 \)) isolated from HBP rats (\( n = 21 \)). The decay of the capacity transient was fitted to a single exponential function, \( y = A_0 \times e^{-t/T} \). Tau was 0.77±0.37 and 0.82±0.36 msec for CTRL and HBP rat heart cells, respectively (\( p = 0.61 \)). The uncompensated series resistance (\( \neq C_m \)) was 4.6±2.3 MΩ for the CTRL rat cells (\( n = 34 \)) and 4.9±2.3 MΩ for the HBP rat cells (\( n = 21 \)) (\( p = 0.61 \)). After compensation, the mean time constant of the capacity transient was shortened to 0.18±0.12 and 0.15±0.05 msec for the CTRL rat cells (\( n = 32 \)) and the HBP rat...
cells (n=18), respectively (p=0.15). The residual series resistance was reduced to 1.2±0.6 MΩ for the normal cells and 1.0±0.4 MΩ for the hypertensive cells (p<0.05). With a maximal inward calcium current of 1,000–3,000 pA peaking in 5 msec, the maximal voltage drop across the residual uncompensated series resistance error was small (about 1.2–3 mV for the CTRL and HBP cells, respectively), and the distortion of the calcium current was minimal.

A previous absolute morphometric study in an identical myocardial hypertrophy rat model showed that the total surface area (sarcolemmal and T tubular) is 1.5–1.6 times the sarcolemmal area for CTRL hearts and 1.7–1.9 times the sarcolemmal area for HBP hearts. Accordingly, the total surface area, based on the calculated apparent surface area, was 1.70–1.81×10⁻⁴ cm² for the CTRL cells and 2.0–2.2×10⁻⁴ cm² for the HBP cells. The specific membrane capacity, defined as the ratio of \( \varepsilon_m \) to the total surface area, was 1.0 to 0.95 \( \mu F/cm² \) for the CTRL cells and 0.84 to 0.77 \( \mu F/cm² \) for the HBP cells. The calculated values for specific membrane capacity in the CTRL and the HBP cells are essentially identical to those reported in all biological "unit" membranes (0.8 to 1 \( \mu F/cm² \)).

The quality of the data obtained using single-pipette, whole-cell voltage-clamp method in this system was examined further by assessing the adequacy of voltage control during activation of \( I_{Ca} \). This was accomplished by terminating the voltage-clamp step around the peak of \( I_{Ca} \). The result of the experiment is illustrated in Figure 2. From a holding potential (\( V_h \)) of \(-40 mV \), voltage steps to 0 mV were applied for 2–16 msec at 2-msec increments. After termination of the voltage steps, the current tails showed a smooth and rapid decay without exhibiting the pattern of an "inverted action potential" or "hooking," which would have indicated poor space clamp control. The above results clearly demonstrate the quality and adequacy of the information.
obtained by use of the single-pipette, whole-cell voltage-clamp method used to characterize $I_{Ca}$.

**Cell Selection**

A major determinant of the amplitude of transmembrane ionic currents is the cell membrane surface area. To minimize the contribution to the amplitude of $I_{Ca}$ of increased cell surface area resulting from hypertrophy and to ensure good voltage-clamp control, smaller cells from HBP rats (but of similar dimensions to those from CTRL rats) were selected for the experiments. Rod-shaped cells were assumed to have the configuration of a right circular cylinder. The diameter and length of the cells were determined at ×600 magnification with a fixed-stage inverted light microscope.

**Results**

**Animal Characteristics**

Voltage-clamp experiments were performed in cells isolated from nine HBP rats and 10 CTRL rats. The mean systolic blood pressure of HBP rats was significantly higher than that of CTRL rats (182±22 vs. 113±12 mm Hg, $p<0.001$). Specific membrane capacitance was determined in 34 CTRL cells and 21 HBP cells. The dimensions for CTRL and HBP cells were 148±15×27±4 μm and 159±14×28±6 μm, respectively. The apparent surface area as calculated for a right circular cylinder was 1.13±0.20×10$^{-4}$ cm$^2$ for the CTRL cells and 1.15±0.18×10$^{-4}$ cm$^2$ for the HBP cells. Another set of 14 CTRL cells and 20 HBP cells was used for voltage-clamp experiments. The dimensions for this set of cells were 148±17×25±2 μm for the CTRL cells and 152±19×27±3 μm for the HBP cells. The apparent surface area was 1.17±0.20×10$^{-4}$ cm$^2$ for the CTRL cells and 1.30±0.28×10$^{-4}$ cm$^2$ for the HBP cells. Because of the cell selection procedure, there was no significant statistical difference in the dimensions and the apparent surface area between these four groups of HBP and CTRL cells (one-way analysis of variance, $p>0.05$).

![Figure 3. Overlapping of the L-type calcium current ($I_{Ca}$) and the transient outward current ($I_{A}$). Panel A: Superimposed current records elicited by 200 msec depolarization pulses from a holding potential of −40 mV to membrane levels indicated to the left in a control cell. Current traces consisted of $I_{Ca}$ and $I_{A}$. Panel B: Blockade of $I_{Ca}$ with 2 mM CdCl$_2$ in the external solution revealed a significant $I_{A}$ in the voltage steps tested. The interrupted line represents the zero current level. Pipette solution contained (mM) KCl 140, MgCl$_2$ 2, CaCl$_2$ 1, EGTA 11, K$_2$ATP 2, HEPES 10 at pH 7.2. External solution contained (mM): NaCl 137, KCl 5.4, MgCl$_2$ 1, CaCl$_2$ 1.8, HEPES 10, glucose 10 at pH 7.4. Cell 88412M3.](http://circres.ahajournals.org/)

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Cell Capacitance and the Capacitative Surface Area

The mean $c_m$ for CTRL cells was $172 \pm 49$ pF ($n=34$) and for HBP cells was $169 \pm 49$ pF ($n=21$). There was no statistical difference between the two groups of cells ($p=0.82$). Assuming a specific membrane capacitance of $1 \mu F/cm^2$ for both CTRL and HBP cells the capacitative surface area was $1.72 \pm 0.49 \times 10^{-4} \text{ cm}^2$ for the CTRL cells and $1.69 \pm 0.49 \times 10^{-4} \text{ cm}^2$ for the HBP cells.

Isolation of $I_{Ca}$

In the plateau range of membrane potentials in rat cardiac myocytes analysis of $I_{Ca}$ is complicated by the overlapping transient outward current, $I_A$. Figure 3A shows superimposed outward current traces in response to various depolarization steps from $V_h=-40 \text{ mV}$, when the internal pipette solution contained 140 mM KCl. The activation and decay of $I_{Ca}$ was in fact distorted by $I_A$. The role of $I_A$ in the range of plateau membrane voltages is illustrated in Figure 3B. Addition of 2 mM CdCl$_2$ to the external solution resulted in the elimination of $I_{Ca}$ and exposure of a large $I_A$. This outward current was activated at $V_m=10 \text{ mV}$ and became progressively larger at higher depolarization steps. Substitution of CsCl for KCl in the pipette solution blocked this current. Figure 4 illustrates the L-type calcium currents obtained after elimination of $I_A$ with CsCl in the pipette solution and under external K$^+$-free conditions and after inactivation of $I_{Na}$ with $V_h=-40 \text{ mV}$.

Effect of Hypertrophy on $I_{Ca}$

Figure 4 shows superimposed records of $I_{Ca}$ in response to selected depolarization steps of 200-msec duration in a CTRL and an HBP myocyte. The amplitude of $I_{Ca}$ was strikingly larger in the HBP cell than in the CTRL cell. The mean current-
voltage relation for 10 CTRL and 15 HBP cells is shown in Figure 5 (upper panel) for comparison. The peak $I_{Ca}$ was larger in the HBP cells than in the CTRL cells at membrane potentials of -20 to 50 mV ($p<0.01$). There was no shift in the current-voltage relation with hypertrophy. The maximal amplitude of $I_{Ca}$ occurred at $V_m = 0$ mV in all 10 CTRL cells. Maximal $I_{Ca}$ was observed at $V_m = 0$ mV in 14 HBP cells and at $V_m = 10$ mV in one HBP cell. To ascertain if the observed increase in the whole cell $I_{Ca}$ is due to a concomitant increase in the total surface area (apparent surface area plus T tubular surface area), the measured peak $I_{Ca}$ magnitude was normalized to the mean cell capacitance determined in 34 CTRL and 21 HBP cells (see above) to obtain the current density. Since the average cell capacitance was virtually identical in these two populations of cells, it is not surprising that the peak $I_{Ca}$ density was larger in the HBP cells than in the CTRL cells. (Figure 5, lower panel).

$I_{Ca}$ recordings in Figure 4 illustrate another major difference between CTRL and HBP cells. Visual inspection of the current traces suggests that the time course of inactivation of $I_{Ca}$ in HBP cells was delayed when compared with CTRL cells. To examine quantitatively the delayed inactivation of $I_{Ca}$, the time course of inactivation of $I_{Ca}$, in response to clamp steps to the plateau range of the action potential was determined in CTRL and HBP cells using a least-squares fit to a two-exponential function of the form

$$A_0 + A_1 \exp(-u/\tau_1) + A_2 \exp(-u/\tau_2)$$

A set of examples of this analysis is shown in Figure 6. Although the fast time constants of inactivation ($\tau_1$) were similar for the CTRL and the HBP cells, the slow time constant of inactivation ($\tau_2$) for the HBP $I_{Ca}$ was clearly slower than that of the CTRL $I_{Ca}$. The mean values for $\tau_1$ and $\tau_2$ for 10 CTRL and 10 HBP cells are shown in Figure 7. There were no significant changes in the fast component of the time course of decay in CTRL and HBP cells. However, the time constant for the slow component of inactivation was longer in HBP than in CTRL cells at $V_m = -10$ to 30 mV with statistical differences ($p<0.05$) demonstrated for $V_m = 0$ and 20 mV.

To compare further the voltage-dependent properties of $I_{Ca}$ in CTRL and HBP cells, the steady-state inactivation curve was determined in four CTRL and four HBP cells. In these experiments, a conventional two-pulse protocol was used. The inactivating prepulse was stepped from a $V_h = -40$ mV to a membrane potential of $-50$ to $+20$ mV in 10-mV steps for 1 second. The test pulse was stepped to $V_m = 0$ mV for 500 msec. The current elicited by the test pulse was expressed as a fraction of the maximal current obtained when the test pulse was preceded by a prepulse hyperpolarized to $-50$ mV ($f_a$). Figure 8 shows $f_a$ plotted against the inactivating prepulse potentials for CTRL and HBP cells. There was no inactivation ($f_a = 1$) of calcium channels at $-40$ mV. $f_a$ for the CTRL cells was represented by

$$f_a(V) = 1/(1 + \exp((V + 28)/4.2))$$

$f_a$ for the HBP cells was represented by

$$f_a(V) = 1/(1 + \exp((V + 27)/4.7))$$

There was no change in the availability of calcium channel for opening in HBP cells because the two inactivation curves were nearly identical.

Calculation of Myoplasmic [Free Ca$^{2+}$] Changes Resulting From Transsarcolemmal Ca$^{2+}$ Influx

From the maximal $I_{Ca}$ traces, the largest change in myoplasmic total calcium (free and bound) concentration, $d[\text{total Ca}]$, due to transsarcolemmal Ca$^{2+}$ influx via $I_{Ca}$ can be calculated from the following equation derived by Fabiato and Baumgarten:

$$d[\text{total Ca}] = \int_0^t (J/v) F(S/v) f/(f_v) dt \quad (1)$$

Figure 5. Upper panel: Mean peak L-type calcium current ($I_{Ca}$) voltage relation for control cells (○) and Goldblatt renovascular hypertensive rat cells (●). $I_{Ca}$ was measured from the holding current level. Lower panel: Peak $I_{Ca}$ current density-voltage relation obtained by normalization of the peak $I_{Ca}$ to the capacitative surface area. $n=10$ and 15 for control and Goldblatt renovascular hypertensive rat cells, respectively. *Significant difference at $p<0.05$. 

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where $t$ is the duration of the fast initial component of $I_{Ca}$, contributing to the activator $Ca^{2+}$ pool(s); $J$ is $I_{Ca}$ density ($coul \times sec^{-1} \times cm^{-2}$); $z$ equals 2, valence for $Ca^{2+}$; $F$ equals 96,500 coul/mol, Faraday's constant; $S/V$ is surface/volume ($cm^{-1}$); $f_{V}$ equals 0.9 at 0 mV, fraction of $I_{Ca}$ carried by $Ca^{2+}$; $f_{V}$ is fraction of cell volume $Ca^{2+}$ enters (fraction of cell volume without mitochondria). On the basis of the absolute morphometric study by Anversa et al. of normal and hypertrophied myocardium from renal hypertensive rats, $S/V$ was calculated to be $0.56 \times 10^{7}$ $cm^{-1}$ for CTRL and $0.64 \times 10^{7}$ $cm^{-1}$ for HBP myocardium, and $f_{V}$ was 0.54 and 0.53 for CTRL and HBP myocardium, respectively. In Table 1, $d[total \ Ca]$ at 10, 15, and 20 msec at 22-25°C is shown. To calculate the myoplasmic [free $Ca^{2+}$] change resulting from $d[total \ Ca]$ the physiological steady state of myoplasmic $Ca^{2+}$ buffering capacity must be considered. According to Fabiato, the major ligands participating in physiological buffering of mammalian ventricular cells include phosphocreatine, calmodulin (with four sequential $Ca^{2+}$ binding sites), the external binding site on SR, the $Ca^{2+}$-specific site of troponin C, the high affinity internal binding site on the sarcolemma, and ATP. Using the apparent stability constants and the myoplasmic concentration of these physiological calcium buffers and the values for $d[total \ Ca]$, the myoplasmic [free $Ca^{2+}$] (expressed as pCa) for CTRL and HBP ventricular myocytes resulting from the transsarcolemmal $Ca^{2+}$ influx was calculated. The results of the calculations are summarized in Table 1.

**Discussion**

**Cardiac Hypertrophy and $I_{Ca}$**

The focus of the present study was on the effect of hypertrophy resulting from gradual chronic pressure-overload on $I_{Ca}$ and its role in the prolongation of the action potential duration. The results
of this study showed that the peak $I_{Ca}$ amplitude in response to clamp steps to the voltage levels of phase 2 and 3 of the action potential was markedly increased in HBP ventricular myocytes. It is unlikely that the observed increase in $I_{Ca}$ results from an increased cell surface area because 1) CTRL and HBP cells of similar lengths and widths were used in the voltage-clamp experiments, and 2) the current density obtained by normalizing $I_{Ca}$ to the capacitative surface area also showed similar striking increases in HBP cells.

Previous studies of the electrical properties of hypertrophied papillary muscle from HBP rat hearts provided indirect evidence suggesting that the lengthened action potential is due to slow inactivation of a Ca$^{2+}$-dependent inward current rather than to a decrease in an outward potassium current or lack of an electrogenic outward current due to reduced activation of Na-K pump.\cite{4,36} The results of the voltage-clamp experiments in the present study confirm this hypothesis. $I_{Ca}$ and $I_{Ca}$ density were increased at all depolarization voltage steps in HBP cells. The time constant of inactivation of the slower component of the maximal $I_{Ca}$ was longer in HBP cells at potentials corresponding to phases 2 and 3 of the rat action potential.

The voltage-dependence of $f_w$ for CTRL cells was identical to that reported in a previous study.\cite{37} The steady-state inactivation of $I_{Ca}$ was not changed in HBP cells, and there was no shift in the voltage step at which the maximal $I_{Ca}$ occurred. Thus, the increased $I_{Ca}$ in HBP cells was not due to a change in the availability of calcium channels for activation in HBP cells.

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**FIGURE 7.** Time constant of inactivation for L-type calcium current at different depolarization potentials in 10 control (○) and 10 Goldblatt renovascular hypertensive rat (●) cells. Upper panel: Mean fast time constant, $\tau_{f\phi}$. Lower panel: Mean slow time constant, $\tau_{s\phi}$. *Significant difference at $p<0.05$.

**FIGURE 8.** Steady-state inactivation of L-type calcium current in four CTRL (○) and four HBP (●) cells. See text for the determination of the inactivation curves. The data points were fitted to the Boltzmann distribution represented by $f_w=1/(1+\exp(V-V_{h}/b))$. Previous Voltage-Clamp Studies on Myocardial Hypertrophy

There has been only one previous voltage-clamp study on the effects of chronic pressure overload on the ionic currents in cardiac tissue.\cite{21} The authors performed single sucrose-gap, voltage-clamp experiments using right ventricular muscle preparations isolated from cats subjected to partial occlusion of the pulmonary artery. $I_{Ca}$ was decreased. There was no difference in the time course of the overall membrane current evoked by depolarization clamp steps in normal and hypertrophied ventricular trabeculae.
The authors concluded that the prolongation of the action potential plateau in the hypertrophied right ventricular trabeculae was due to a reduction in the background and potassium outward currents. These results are opposite to the findings in the present study. These discrepancies may at least be partially explained by 1) the limitations inherent in the sucrose-gap, voltage-clamp method using ventricular trabeculae and 2) differences in the animal model of hypertrophy and the degree of myocardial dysfunction.

\( I_{Ca} \) in Excitation-Contraction Coupling

An increased and slowly inactivating \( I_{Ca} \) in HBP cells will alter myocardial contractility 1) by prolonging the plateau phase of the action potential, and/or 2) by increasing the transmembrane flux of activator \( Ca^{2+} \) for excitation-contraction coupling. Although evidence for a direct correlation between \( I_{Ca} \) and contractility is lacking, results from several voltage-clamp studies suggest a close relation between \( I_{Ca} \) and contractility in ventricular muscles.\(^{12,14-18}\) Transmembrane influx of calcium ions via \( I_{Ca} \) plays a key role in the process of \( Ca^{2+} \)-triggered release of calcium from the SR to initiate contraction in mammalian hearts.\(^{20}\) In this connection, although there are no available data on the activator calcium requirement in hypertrophied myocardium, the increased \( I_{Ca} \) and prolonged action potential duration in HBP myocardium are in agreement with the supposition that these changes represent a compensatory mechanism for impaired activation of contractility. Although the pCa levels achieved with an increased \( I_{Ca} \) in HBP cells are insufficient to activate contraction directly, in the scheme of the calcium-triggered calcium release hypothesis such an increase would 1) provide an increased amount of activator calcium to compensate for a "blunted" release of \( Ca^{2+} \) from SR as occurs in aging rat myocardium;\(^{36,39}\) and 2) in the presence of a normal \( Ca^{2+} \) release mechanism, the increased activator \( Ca^{2+} \) would serve to amplify the release of \( Ca^{2+} \) from SR, thereby providing more \( Ca^{2+} \) to bind to the increased myofibrillar volume in hypertrophied myocardium.\(^{19}\) The overall goal of preserving myocardial contractility in hypertrophied cardiac muscles would thus be achieved by this putative compensatory mechanism.

Voltage-Clamp Technique

The single-electrode, whole-cell patch-clamp method minimizes some of the limitations and difficulties inherent in multicellular preparations.\(^{22,23,25}\) This is of great importance in the characterization of \( I_{Ca} \) in rat myocardium because of the overlapping of \( I_{Ca} \) with \( I_{A} \) and the delayed outward current (Figure 3). The whole-cell, patch-clamp method allows the separation of \( I_{Ca} \) from these currents by replacement of intracellular \( K^{+} \) with \( Cs^{+} \) via the suction pipette (Figure 4).\(^{39-41}\)

Use of enzymatically isolated single ventricular cells may, on the other hand, introduce other problems. Since not all cells recover after enzymatic dissociation, there is the potential sampling bias toward those cells that survived. This bias could be further complicated by the possibility that a different subpopulation of cells survived from the hypertrophied rat heart and by a substantial overlap in the size of cells from the normal and hypertrophied hearts. The voltage-clamp experiments show that distinctively different electrical properties can be identified in single ventricular myocytes enzymatically isolated from CTRL and HBP rat hearts. This is in agreement with results reported by Aronson and Nordin.\(^{10}\) These authors, using a similar collagenase isolation technique, were able to demonstrate isolation of hypertrophied myocytes, as confirmed by significant increases in cell length and width, from HBP rat hearts. Moreover, characteristic electrical properties were preserved during the enzymatic isolation process since the transmembrane action potentials recorded from their HBP cells show similar increases in the duration of action potential as in the intact tissue.\(^{3}\) Thus, the results of the voltage-clamp experiments in the present study are unlikely to be due to sampling bias or artifacts introduced by the enzymatic isolation process. Electrophysiologic alterations in diseased myocardium can be investigated with the whole-cell, patch-clamp technique using enzymatically isolated single cardiac myocytes.
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