Calcium Current in Single Cells Isolated From Normal and Hypertrophied Rat Heart

Effects of β-Adrenergic Stimulation

F. Scamps, E. Mayoux, D. Charlemagne, and G. Vassort

The L-type calcium current was investigated in normal and hypertrophied rat ventricular myocytes as a possible cause of the action potential lengthening that has been reported during hypertrophy. Regulation of the calcium current (I\textsubscript{Ca}) by a β-adrenergic agonist (isoproterenol) was also analyzed since β-agonist-induced positive inotropy is less marked in hypertrophied heart. Left ventricular hypertrophy was induced by stenosis of the abdominal aorta. For recording I\textsubscript{Ca}, the whole-cell patch-clamp technique was used. Potassium currents were suppressed by replacing K\textsuperscript+ ions with Cs\textsuperscript+ ions in both the extracellular and intracellular media, and sodium current was blocked by 50 μM tetrodotoxin. The Ca\textsuperscript{2+} current was larger in hypertrophied cells (2.2±0.6 nA [n=31]) than in normal cells (1.2±0.5 nA [n=33]). However, if one relates I\textsubscript{Ca} amplitude to the cell membrane area, as estimated by membrane capacitance measurement, no significant difference was observed in current density (8.5±2.5 pA/pF [n=31] and 8.3±2.1 pA/pF [n=33] in hypertrophied and in normal cells, respectively). In both cell types, I\textsubscript{Ca} displayed the same voltage and time dependence. When expressed as a percentage, the maximal increase in I\textsubscript{Ca} amplitude that was obtained with 100 nM isoproterenol was less in hypertrophied cells (+78%) than in normal cells (+120%). The sensitivity of I\textsubscript{Ca} to β-adrenergic stimulation was not modified: EC\textsubscript{50} was 3.8 nM for hypertrophied cells and 4.8 nM for normal cells. Forskolin and cyclic AMP were as effective in both cell types. Stimulation of I\textsubscript{Ca} by β-adrenergic agonist was decreased in agreement with a reduced number of binding sites of β-agonists and/or an altered coupling of the G-proteins. (Circulation Research 1990;67:199–208)

Chronic overloading of the heart induces hypertrophy of the myocardium, leading to structural, biochemical, and functional alterations of the myocardial cells. The depressed contractility state of hypertrophied hearts might involve the excitation-contraction coupling process in which the transsarcolemmal Ca\textsuperscript{2+} influx is a key element. Prolonged action potential duration is a general property of hypertrophied hearts in different species. This has also been shown in myocytes isolated from hypertrophied hearts. Some experiments with multicellular preparations have attributed this prolongation to a slower inactivation of the slow inward current. This was reinforced by the observation that on isolated cells the time constant of the slow component of calcium current (I\textsubscript{Ca}) inactivation is increased. Besides changes in kinetics, the amplitude of the ionic currents underlying action potential could also be affected. A previous study by Ten Eick et al suggested that the magnitude of both the slow inward current and late outward current is decreased in the papillary muscle of cats with pressure overload–right ventricular hypertrophy. A more recent analysis on the same model conducted on isolated cells extends this work and suggests that there is no change in I\textsubscript{Ca} density and a decrease in outward current. On the other hand, a 2.5-fold increase in peak I\textsubscript{Ca} density is shown in Goldblatt renovascular hypertensive rats. Information about calcium channel density is also available from nitrindipine binding studies. Hypertension decreased the number of calcium channel receptor binding sites in the Goldblatt rat model, increased it in old spontaneously hypertensive rats, and did not modify it in young spontaneously hypertensive rats. On the other hand, similar densities are reported in normal and hypertrophied rats after aortic stenosis. Besides changes in transmembrane calcium flux in hypertrophied myocardium, an altered response to

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inotropic agents could also be due to a modification of the sarcoplasticum receptor function. Contradictory results have been reported after β-adrenergic stimulation with differences related to the model of hypertrophy and the level of circulating catecholamines. In the hypertrophied rat heart after abdominal aortic constriction, as used in this study, a depressed responsiveness to isoproterenol has been reported.\(^{15}\)

In the present study using whole-cell patch-clamp, we compared the characteristics of \(I_{\text{Ca}}\) in normal and hypertrophied left ventricular rat cells after aortic stenosis. The relation between depressed β-adrenergic inotropic response and \(I_{\text{Ca}}\) modulation in the rat hypertrophied myocardium was also investigated.

**Materials and Methods**

**Aortic Stenosis**

Surgery was performed on male Wistar rats (180–200 g). They were fed ad libitum with M25 biscuits (Extra Labo, Piteamment, Provins, France) until operated or killed. Rats were anesthetized by an intraperitoneal injection of 0.05 mg·g\(^{-1}\) sodium pentobarbital. Abdominal aortic stenosis was induced using Weck forceps (Edward Weck & Co., Research Triangle Park, N.C.) modified according to the method of Cutileta et al\(^{16}\); each operated rat was paired with a sham-operated rat of the same weight. The rats were killed for experiments 4–5 weeks after the surgery.

Since hearts had to be kept intact to undergo the digestion procedure used to isolate myocytes, the total wet weight of the heart was taken into account to estimate the hypertrophy. The heart was rapidly extracted from the animal and rinsed in cold saline solution. Excess mediastinal tissues were removed; the heart was then placed into a tared beaker containing cold saline solution. Only hypertrophied hearts with a heart weight/body weight ratio greater than 30% compared with the ratio of sham-operated hearts were selected.

**Cell Preparation**

Myocytes were isolated according to the method of Wittenberg et al.\(^{17}\) At the end of the collagenase perfusion, a piece of left ventricle was cut off and stirred to obtain cells. The isolated cells were centrifuged through Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) to increase the proportion of intact myocytes (90%). The cells were suspended in tissue culture in Petri dishes at 37°C. These cells were used within 10 hours of their isolation.

**Solutions**

The cells in a tissue culture dish were superfused by gravity with a solution containing (mM) CsCl 20, NaCl 117, CaCl\(_2\) 1.8, MgCl\(_2\) 1.7, NaH\(_2\)PO\(_4\) 1.5, NaHCO\(_3\) 4.4, glucose 10, and HEPES 10, pH 7.4, at 20°C bubbled with 100% O\(_2\). Once a cell was sealed to the electrode, it was exposed to different extracellular solutions by positioning it at the extremity of one of six capillaries. These capillaries contained either the solution described above to which 50 μM tetrodotoxin had been added to block the sodium current or solutions containing different concentrations of active compounds. (+)-Isoproterenol, forskolin, cyclic AMP, cadmium chloride, 4-aminopyridine, and tetrodotoxin were obtained from Sigma; ryanodine was obtained from Calbiochem Corp., La Jolla, Calif.

The internal solution in the patch electrode (0.6–1.5 MΩ) contained (mM) CsCl 120, MgCl\(_2\) 4, Na\(_2\)ATP 3, Na\(_2\)-creatine phosphate 5, Na\(_2\)GTP 0.4, K\(_2\)EGTA 5, CaCl\(_2\) 0.062 (intracellular free calcium concentration of 10\(^{-9}\) M), and HEPES 10, adjusted to pH 7.1 with KOH (total intracellular potassium of 20 mM).

**Experimental Arrangements and Data Analysis**

The methods used for whole-cell patch-clamp experiments and for the analysis of data were derived from those developed on single frog cells.\(^{18}\) For monitoring \(I_{\text{Ca}}\), ventricular cells were depolarized from −70 mV holding potential to 0 mV for 200 msec every 4 seconds with a patch-clamp amplifier (model RK-300, Biologic, France). \(I_{\text{Ca}}\) was measured on-line as the difference between peak inward current and the current at the end of the 200-msec pulse. Currents were digitized at 10 kHz (12-bit A/D converter) and analyzed on-line with programs in PASCAL by a Compaq 286 Desk-Pro computer (COMPAQ Computer Corp., Houston). Just after the patch was broken, pulses from −81 to −79 mV were applied to the cell. The exponential components of the decaying current were determined and analyzed by a program that uses the Padé-Laplace method.\(^{19,20}\) Two time constants were obtained corresponding to the electrode and the membrane capacitance. The time constant of electrode capacitance was 0.02 msec or less, and the time constant of membrane capacitance was 1 msec or less. After compensation of electrode capacitance, the capacitance of the membrane was calculated according to the equation \(C_{\text{m}} = T_e \cdot I_e / \Delta E_{\text{m}} [1 - (I_e / I_c)]\), where \(C_{\text{m}}\) is membrane capacitance, \(T_e\) is the time constant of the membrane capacitance, \(I_e\) is maximum capacitance current value, \(\Delta E_{\text{m}}\) is the amplitude of voltage step, and \(I_c\) is the amplitude of steady-state current\(^ {21}\) (negligible in our conditions). The series resistance was calculated as \(R_s = \Delta E_{c} / I_c\), where \(R_s\) is series resistance, and ranged from 2 to 6 MΩ. Membrane capacitance and series resistance were not compensated. The kinetics of \(I_{\text{Ca}}\) were analyzed using the computer program EXCALC.\(^ {20}\) Statistical analysis was performed with the computer program STATGRAPH (STSC Inc., Rockville, Md.). Values are expressed as mean±SD.

**Results**

**Animal and Cell Characteristics**

Body weight of stenosed rats did not differ from that of sham-operated rats (Table 1). Heart weight of stenosed rats selected for this study was significantly higher (+48.5%) than that of the sham-operated rats, which is in agreement with the expected left
ventricular hypertrophy. The heart weight/body weight ratio (in milligrams per gram) was significantly larger in the stenosed group than in the sham-operated group, as expected. Chronic heart failure never occurred in this model, as confirmed by anatomic examination of the lungs and liver.

Cells isolated from hypertrophied hearts were significantly larger than those from sham-operated hearts (Table 1). The myocyte surface area, assuming a cylindrical cell shape in which the diameter corresponds to the width, was significantly larger in hypertrophied hearts (+47.8%) than in control hearts. The similar increases in heart weight/body weight ratio and myocyte surface area clearly show that hypertrophy occurred at the cellular level.

**Action Potentials**

In this model of hypertrophy by aortic stenosis, an increase in action potential duration of ventricular fibers was already reported. However, no results are available on isolated cells. Figure 1 shows recordings of action potentials from normal and hypertrophied ventricular cells obtained in 5.4 mM extracellular potassium and 140 mM intracellular potassium, instead of cesium, with no change in the other ions or metabolites (see “Materials and Methods”). The action potentials from normal cells displayed the same characteristics as generally recorded with a patch electrode containing EGTA, that is, a short action potential with suppression of the late plateau. Table 2 summarizes the mean values of action potential duration measured at 25%, 50%, 75%, and 90% of repolarization in normal and hypertrophied cells. A significant increase in the action potential duration of hypertrophied rat hearts was observed at all levels of repolarization. Action potential amplitude was not significantly modified nor was the resting potential. Since repolarization of the action potential is the result of a fine balance between the outward and inward currents and since I₉ is known to play a major role at the plateau level, changes in I₉ amplitude and/or kinetics could account for the above results.

**Recording of the Calcium Current**

Current recordings were performed in the presence of tetrodotoxin and cesium and were compared with those obtained after the addition of 2 mM Cd²⁺. Subtraction of the cadmium-resistant current from the total current gives a slow inward current (Figure 2A). Current-voltage relations were established in both control and cadmium-containing solutions for the maximal inward current (Figure 2B) and for the current at the end of the 200-msec pulse (Figure 2C). Figure 2B shows that the cadmium-sensitive current and the inward current, estimated by the difference between the peak inward current and the current at the end of the 200-msec pulse, have a similar amplitude and voltage dependence. Analysis on an expanded scale of the current at 200 msec reveals that it had a rather complex voltage dependence, whereas the cadmium-resistant current increased smoothly with voltage. The cadmium-sensitive, bimodal, inward current at 200 msec was sometimes superimposed on a small outward current that increased with increasing depolarizations. Rundown of peak Iₗ was very limited, generally less than 10% within 30 minutes. Cells showing pronounced rundown were discarded. In the following experiments, for practical reasons, the inward current attributed to calcium entry, I₉, was generally estimated by the difference between the peak inward current and the current at the end of the pulse.

**Table 1.** Body Weights, Heart Weights, and Cell Characteristics of Control and Hypertrophied Rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>Body wt (g)</th>
<th>Heart wt (g)</th>
<th>Heart wt/body wt (mg/g)</th>
<th>Cell length (µm)</th>
<th>Cell width (µm)</th>
<th>Cell area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>298±46</td>
<td>1.01±0.16</td>
<td>3.46±0.32</td>
<td>115±16</td>
<td>27.5±7.5</td>
<td>11,298±4,043</td>
</tr>
<tr>
<td>n = 17</td>
<td>n = 17</td>
<td>n = 17</td>
<td>n = 32</td>
<td>n = 32</td>
<td>n = 32</td>
<td>n = 32</td>
</tr>
<tr>
<td>Hypertrophied</td>
<td>306±30</td>
<td>1.50±0.19*</td>
<td>4.84±0.42*</td>
<td>134±2†</td>
<td>35.0±7.0†</td>
<td>16,705±4,082</td>
</tr>
<tr>
<td>n = 19</td>
<td>n = 19</td>
<td>n = 19</td>
<td>n = 27</td>
<td>n = 27</td>
<td>n = 27</td>
<td>n = 27</td>
</tr>
</tbody>
</table>

Values are mean±SD. At most, three cells were studied from each dissociation. Normal, control sham-operated rats; hypertrophied, rats with forceps-induced abdominal aortic stenosis; n, number of cells.

*P<0.001 compared with normal rats.

†P<0.01 compared with normal rats.

**Figure 1.** Action potentials recorded in single cells isolated from normal (N) and hypertrophied (H) adult rat hearts.
Several experiments were performed in the presence of 2 mM 4-aminopyridine or 10 μM ryanodine (preceded by 15–60 minutes of incubation at 37°C) without changes in current amplitude, time course, or voltage dependence. A few other experiments were performed with the holding potential fixed at −50 mV. Once more, the inward current had a similar time course and voltage dependence, but its amplitude was slightly decreased. Figure 2B shows the difference between the currents obtained on a cell held at −70 mV and then at −50 mV for different membrane potentials. This difference in amplitude could be attributed to a slow rundown of the calcium conductance or to a very small decrease in its availability.

### Characteristics of ICa in Control and Hypertrophied Hearts

Figure 3A shows calcium current-voltage relations and two superimposed ICa tracings obtained from normal and hypertrophied cells. These tracings illustrate the difference in ICa amplitude recorded on the two cell types. Both currents displayed the same voltage dependence. The threshold potential of activation was −38.5±6.1 (n=16) and −39.3±5.3 (n=19) mV for normal and hypertrophied cells, respectively. The potential for maximal activation was −0.9±4.0 (n=16) and +1.0±5.7 (n=19) mV; the estimated reversal potential (after linear extrapolation of the positive slope conductance) was +45.9±4 (n=16) and +46.0±3.2 (n=19) mV for normal and hypertrophied cells, respectively.

The mean values of membrane capacitance and ICa amplitude and density obtained under similar conditions in cells isolated from normal and hypertrophied rat hearts are given in Table 3. The significant increase in membrane capacitance (+78%) was in agreement with hypertrophy of myocytes, as were cell dimensions. ICa amplitude of hypertrophied cells was also significantly larger (+88%). However, since membrane capacitance and ICa amplitude increased in the same order, the current density expressed by the ICa/membrane capacitance ratio was similar in both cell types. The current density-voltage relations are superimposable (Figure 3B).

A kinetic analysis was performed on ICa elicited by varying membrane depolarization. Most generally two components were detected so that ICa could be described by the equation:

$$I_{Ca} = A_1 \cdot e^{-v_a} + A_3 \cdot e^{-v_b} + A_{sh}$$
Calcium Current in Normal and Hypertrophied Heart

FIGURE 3. Panel A: Graph showing current-voltage relations established for the calcium current (I_{Ca}) elicited by depolarization from a holding potential of −70 mV in normal (◼), peak I_{Ca} measured as the difference of the peak inward current and the steady-state current; △, current at the end of the 200-msec depolarization, relative to the zero current) and in hypertrophied (●), peak I_{Ca}; +, steady-state current) cells. Basal stimulation frequency was 0.25 Hz. Inset: Superimposed current tracings obtained during depolarizations from −70 to 0 mV for 200 msec in normal (N) cell (membrane capacitance of 133 pF) and in hypertrophied (H) cell (membrane capacitance of 271 pF). Panel B: Current-voltage relations of normalized I_{Ca} to membrane capacitance from 16 normal (◼) and 19 hypertrophied (●) cells.

TABLE 3. Calcium Current in Isolated Rat Cells From Control and Hypertrophied Heart

<table>
<thead>
<tr>
<th>Cells</th>
<th>C_m (pF)</th>
<th>I_{Ca} (pA)</th>
<th>I_{Ca}/C_m (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>148.1±40.3</td>
<td>1,197±514</td>
<td>8.27±2.15</td>
</tr>
<tr>
<td>Hypertrophied</td>
<td>264.1±76.3*</td>
<td>2,254±651*</td>
<td>8.51±2.47</td>
</tr>
</tbody>
</table>

Values are mean±SD. C_m, membrane capacitance; I_{Ca}, amplitude of calcium current elicited by depolarizing the cell at 0 mV from −70 mV holding potential; I_{Ca}/C_m, current density; normal, cells from control sham-operated rats; hypertrophied, cells from rats with forceps-induced abdominal aortic stenosis. Thirty-three cells were taken from 15 normal hearts; 31 cells were taken from 15 hypertrophied hearts.

*p<0.001 compared with normal cells.

inward current voltage relation, whereas the voltage dependence of A_s and \( \tau_s \) clearly shifted toward lower depolarizations. The physiological meaning of these two components is as yet unclear.

Steady-State Activation and Inactivation and Frequency-Dependent Effects of I_{Ca}

Steady-state activation and inactivation voltage dependencies of the inward current recorded in control and hypertrophied cells are reported in Figure 5. No difference was observed in either the potential giving 50% of inactivation (−23.4±4.3 mV [n=16] for normal cells and −26.5±4.5 mV [n=19] for hypertrophied cells) or in the relief from inactivation after prepulse at +50 mV (43.8±9.8% [n=16] for normal cells and 41.6±10.6% [n=19] for hypertrophied cells). Notice that the product of the steady-state activation and inactivation curves above −35 mV is never null; this implies the flow of an inward window current between this potential and the calcium reversal potential. The reactivation process was studied by a standard two-pulse protocol. Figure 6A shows two typical reactivation curves. An overshoot was generally observed with a pulse interval between 400 msec and 1.2 seconds, suggesting a phase of hyperexcitability. The times for 50% of reactivation were 89.0±18.6 (n=8) and 102.2±17.4 (n=5) msec for the normal and hypertrophied cells, respectively. The difference was not significant. Figure 6B illustrates the effects of increasing the stimulation frequency on the amplitude of I_{Ca} in normal and hypertrophied cells (control frequency was 0.25 Hz). In both, on switching to a higher frequency, the amplitude of I_{Ca} first increased and then decreased. The percentages of decrease near steady state at 1, 1.6, and 2.5 Hz were 7.4±4.6%, 17.8±6.4%, and 34.0±5.6%, respectively, in normal cells (n=6) and 11.9±6.6%, 24.5±1.7%, and 40.2±6.7%, respectively, in hypertrophied cells (n=4); the differences were not significant.

Effect of \( \beta \)-Adrenergic Stimulation on I_{Ca}

We have also investigated the effects of \( \beta \)-adrenergic stimulation. Figure 7 shows the doseresponse curves obtained on normal and hypertrophied cells. At most, three cumulative doses of

where A_f, A_s, and A_m are the amplitudes of the fast, slow, and steady-state components, respectively, and \( \tau_f \) and \( \tau_s \) are the time constants of the fast and slow components, respectively. Results are shown in Figure 4. There was no significant difference in the three sets of values obtained in control or in hypertrophied cells when the amplitudes were expressed in densities of current per surface area. Both components exhibited voltage dependence of their time constant and amplitude at zero time; however, the voltage dependence of A_s and \( \tau_s \) roughly followed the maximal
isoproterenol were used for the lowest concentrations (from 0.1 to 10 nM). No cumulative doses were used with the highest concentrations to avoid desensitization. The threshold of the \( \beta \)-adrenergic effect appeared below 1.0 nM, and the maximal effect was obtained with 100 nM isoproterenol. A maximally active isoproterenol concentration increased \( I_{Ca} \) as much as twofold in normal cells within 2 minutes (+120%). Isoproterenol was significantly less efficient in hypertrophied cells (+78%). However, the potency (EC\(_{50}\)) of isoproterenol to increase \( I_{Ca} \) was similar in normal and hypertrophied cells; the EC\(_{50}\) was 4.8 nM in normal cells and 3.8 nM in hypertrophied cells. Otherwise, in both cell types, there were no changes in either time constants of \( I_{Ca} \) inactivation or the \( I_{Ca} \) availability curves by the \( \beta \)-adrenergic stimulation (not shown).

The difference in the responses elicited by \( \beta \)-adrenergic stimulation in the normal and hypertrophied cells could be due, among other activities, to a decrease in the intrinsic activity of the adenylate cyclase and/or in the phosphorylation of the calcium channel by protein kinase A. To check these hypotheses, the effects of external application of 10 \( \mu \)M forskolin and of internal dialysis of 50 \( \mu \)M cyclic AMP were investigated on both cell types. The maximal responsiveness of \( I_{Ca} \) to isoproterenol, cyclic AMP, and forskolin is illustrated in Figure 8. The
percent increase is given relative to current density of control, normal, and hypertrophied cells. In normal myocytes, isoproterenol, cyclic AMP, and forskolin led to about a 120% increase in \( I_{Ca} \). In hypertrophied cells, cyclic AMP and forskolin had the same relative effect as in control cells; they were significantly more efficient than isoproterenol stimulation.

### Discussion

The aim of this study was to characterize and compare \( I_{Ca} \) in normal and hypertrophied rat hearts to clarify its role in the prolongation of action potentials associated with hypertrophy. Under our experimental conditions, hypertrophy was induced by pressure overload after aortic stenosis. To be sure that myocytes had been subjected to pressure overload, only cells from the left ventricle were studied. The hypertrophy of the cell was estimated by two methods: determination of cell dimensions and measurement of cell capacitance. In agreement with the morphological studies of Anversa et al., our cells, isolated from hypertrophied hearts, had a larger surface area. The hypertrophied state of the myocytes was also confirmed by a larger membrane capacitance. Assuming a specific membrane capacitance of 1 \( \mu \)F/cm², the capacitive surface areas for normal and hypertrophied cells were \( 1.48 \times 10^{-4} \pm 0.40 \times 10^{-4} \) cm² and \( 2.64 \times 10^{-4} \pm 0.76 \times 10^{-4} \) cm², respectively. Lower values of surface area were obtained when calculated from the dimension of cells measured visually (\( 1.13 \times 10^{-3} \pm 0.40 \times 10^{-4} \) cm² and \( 1.67 \times 10^{-4} \pm 0.41 \times 10^{-4} \) cm² for normal and hypertrophied myocytes, respectively). These discrepancies between the two methods could be related to the membrane invaginations, such as caveolae and the T-tubular system, which are not taken into account with the morphometric measurements. The capacitive surface area/morphometric surface area ratio was 1.3 and 1.6 for normal and hypertrophied cells, respectively, and could reflect the percentage of membrane invaginations. These values are close to those previously reported and confirm the higher proportion of invaginated membrane in hypertrophied myocytes.

This study reports a prolongation of the action potential of isolated hypertrophied cells after aortic banding. Previous studies on other models also showed prolongation of action potential of hypertrophied isolated myocytes. In our experimental conditions, recordings of action potentials were made with a patch electrode containing EGTA and allowed control of the internal medium, which suggests that direct changes of ionic conductances may trigger the above effects.

We have compared \( \text{Ca}^{2+} \) currents recorded under whole-cell patch-clamp techniques in normal and
hypertrophied cardiac rat cells. The slow inward current measured under whole-cell conditions might be described as a composite waveform consisting of at least two major components, T-type and L-type ICa,s, which flow through two types of calcium channels.\textsuperscript{25,26} As indicated by the voltage dependence, only the L-type Ca\textsuperscript{2+} current could be recorded at the routinely used membrane holding potential (−70 mV) in both cell types. Similar results have been reported with the use of a more negative holding potential.\textsuperscript{27} Furthermore, there was a sustained, cadmium-sensitive component that exhibited complex voltage dependence. This component had a low amplitude, that is, a small percentage of maximal ICa. This observation is in agreement with previous observations.\textsuperscript{26,28} It could reflect an incomplete inactivation of ICa consequent to the 200-msec pulse duration we used, to the nonspecific background current, or to the window current as supported by the steady-state activation and inactivation voltage dependence of ICa (Figure 5). In fact, the expected window current would be less than 5% of maximal peak current at −20 mV and much less above −5 mV because either the product of the steady-state activation and inactivation curves is small in the −5 to +10 mV range or the driving force is reduced for more positive potentials. This further justifies our experimental procedure in which ICa is estimated at 0 mV, the potential at which the contributions of the window current and the cadmium-resistant current are minimal (Figures 2 and 5A). In several mammalian cardiac cells, a transient outward current is elicited by depolarizations positive to −20 mV.\textsuperscript{30,31} There are several reasons to believe that this current has been suppressed by the cesium ions under our basal conditions since the calcium current-voltage relations were unaffected by 4-aminopyridine and ryanodine or by holding the membrane at −50 mV, a condition that inactivates it by half. Voltage characteristics of ICa in normal rat cells are in agreement with previously published data.\textsuperscript{28,29,32} With a “gapped” two-pulse protocol, relief from inactivation was observed at positive potentials as described on the same preparation by Josephson et al.\textsuperscript{29} However, this result is not in agreement with the one obtained by Cohen and Lederer\textsuperscript{28} on rat ventricular cells. This could result from a higher frequency of stimulation in their case as initially pointed out by Lee et al.\textsuperscript{33} The behavior of ICa was modulated by the stimulation frequency. An increase in stimulation frequency (>0.25 Hz) induced a maintained negative staircase that was always preceded by a transient positive staircase of ICa, in agreement with the overshoot on the reactivation curve. To date, both negative and positive staircases have been reported when the
of calcium channels are not characteristics of calcium channels are not modified by hypertrophy. In fact, I_{Ca} amplitude of single hypertrophied myocytes was larger than in normal myocytes; this increase is correlated with the larger membrane capacitance. Thus, when peak I_{Ca} was normalized to the cellular membrane capacitance, estimated under voltage-clamp conditions, current density was not modified. To date, only two reports in isolated myocytes deal with the comparative analysis of I_{Ca} also normalized to membrane capacitance. Kleiman and Houser\(^6\) found no change in peak I_{Ca} density of feline myocytes while Keung\(^5\) found a 2.5-fold increase in Goldblatt hypertrophied rat myocytes. The discrepancy between the results of Keung and others may be a consequence of the different hypertrophy models. A tentative explanation for discrepancy could be that hypertrophy induces first a synthesis of calcium channels and second an increase in cell size, which the selection of cells revealed. In our model, synthesis of calcium channels would be concomitant with the increase in cell surface area since the dihydropyridine-receptor density\(^4\) and the inotropic response of Ca\(^{2+}\) and calcium channel modifying agents\(^6\) were unchanged.

The shortening of the action potential could also be due to alterations in the kinetics of I_{Ca}. Our study shows that the two time constants of I_{Ca} inactivation are identical in normal and hypertrophied cells in accordance with the results of Ten Eick et al\(^10\) concerning the hypertrophied right ventricle of the cat, but not with the results of Kleiman and Houser\(^6\) and Keung\(^5\) concerning the slow time constant. Furthermore, inactivation, reactivation, and frequency dependence of I_{Ca} were similar in both cell types in accordance with previous reports.\(^6\),\(^9\) On the other hand, biochemical alterations of the intracellular calcium regulation and internal ATP concentration reported in the hypertrophied heart\(^2\),\(^3\),\(^7\),\(^8\) could account in part for the altered electrical properties in modifying, for example, the calcium permeability and kinetics of I_{Ca} of the hypertrophied cells. However, even when both cell types were identically perfused by the patch pipette, differences in action potential durations were seen. Thus, our results lead us to conclude that calcium channels of hypertrophied cells display the same electrical properties as those of normal cells.

As previously reported,\(^3\) \(\beta\)-adrenergic stimulation in normal rat cells by isoproterenol increases I_{Ca} twofold without alterations in kinetic parameters of I_{Ca}. In hypertrophied cells, maximal doses of isoproterenol were less effective. Our results, showing no change in the sensitivity of I_{Ca} to isoproterenol, agree with the lack of modification of receptor affinity in hypertrophied cells.\(^3\) Moreover, experiments with cyclic AMP and forskolin suggest that adenylate cyclase activity or phosphorylation by protein kinase A are unaltered during hypertrophy. Finally, the reduced responsiveness to \(\beta\)-adrenergic agents showed a clear correlation with the lower number of \(\beta\)-adrenoceptors reported in the rat aortic model of stenosis.\(^15\) Possible interpretations could be alterations in externalization and/or internalization of the \(\beta\)-adrenoceptors or loss of high-affinity receptors consequent to deficiency of the guanosine nucleotide regulatory proteins as reported in pressure-overload left ventricular failure.\(^40\)

Therefore, we suggest that the shortened action potential and the depressed contractility state recorded in hypertrophied rat heart after aortic stenosis cannot be accounted for by alterations in the calcium channel. However, a major modification seems to occur in the regulation of the calcium channel by the \(\beta\)-adrenergic system as a consequence of a reduced number of receptors and/or an altered coupling of the G-proteins. Thus, our results suggest that the abnormalities in the response to inotropic drugs during chronic cardiac hypertrophy are more likely to be explained by changes in specific receptors than by a reduction in calcium channels.

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References


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