Mechanisms of L-Type Ca\textsuperscript{2+} Current Downregulation in Rat Atrial Myocytes During Heart Failure

Christophe Boixel, Walter Gonzalez, Liliane Louedec, Stéphane N. Hatem

Abstract—Downregulation of the L-type Ca\textsuperscript{2+} current (\(I_{\text{Ca}}\)) is an important determinant of the electrical remodeling of diseased atria. Using a rat model of heart failure (HF) due to ischemic cardiopathy, we studied \(I_{\text{Ca}}\) in isolated left atrial myocytes with the whole-cell patch-clamp technique and biochemical assays. \(I_{\text{Ca}}\) density was markedly reduced (1.7±0.1 pA/pF) compared with sham-operated rats (S) (4.1±0.2 pA/pF), but its gating properties were unchanged. Calcium channel \(\alpha_{\text{IC}}\)-subunit quantities were not significantly different between S and HF. The \(\beta\)-adrenergic agonist isoproterenol (1 \(\mu\)mol/L) had far greater stimulatory effects on \(I_{\text{Ca}}\) in HF than in S (2.5- versus 1-fold), thereby suppressing the difference in current density. Dialyzing cells with 100 \(\mu\)mol/L cAMP or pretreating them with the phosphatase inhibitor okadaic acid also increased \(I_{\text{Ca}}\) and suppressed the difference in density between S and HF. Intracellular cAMP content was reduced more in HF than in S. The phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine had a greater effect on \(I_{\text{Ca}}\) in HF than in S (76.0±11.2% versus 15.8±21.2%), whereas the inhibitory effect of atrial natriuretic peptide on \(I_{\text{Ca}}\) was more important in S than in HF (54.1±4.8% versus 24.3±8.8%). Cyclic GMP extruded from HF myocytes was enhanced compared with S (55.8±8.0 versus 6.2±4.0 pmol · mL\textsuperscript{−1}). Thus, \(I_{\text{Ca}}\) downregulation in atrial myocytes from rats with heart failure is caused by changes in basal cAMP-dependent regulation of the current and is associated with increased response to catecholamines. (Circ Res. 2001;89:607-613.)

Key Words: heart failure ■ L-type Ca\textsuperscript{2+} current ■ atrial fibrillation ■ rat atrial myocytes

In fibrillating\textsuperscript{1,2} or hemodynamically overloaded atria\textsuperscript{3} and during heart failure (HF),\textsuperscript{4} the action potential of atrial myocytes is short, loses its notch and plateau phase, and adapts poorly to changes in heart rate. It is now generally agreed that this cellular electrical remodeling contributes to the occurrence and perpetuation of atrial fibrillation. A number of authors have attempted to identify the molecular mechanisms underlying the action potential alteration in both human and animal tissues. The results point to a reduction in the L-type Ca\textsuperscript{2+} current as one of the main mechanisms responsible for action potential shortening.\textsuperscript{1-4} For instance, a drastic 70% reduction in the calcium current has been reported in human myocytes from dilated\textsuperscript{3} or chronically fibrillating\textsuperscript{1} atria.

\(I_{\text{Ca}}\) downregulation could be caused by a variety of mechanisms. For instance, there is biochemical evidence that sustained atrial fibrillation (AF) and rapid pacing both cause a reduction in calcium channel \(\alpha_{\text{IC}}\)-subunit expression.\textsuperscript{5-10} The effects of \(\beta\)-adrenergic agonists on \(I_{\text{Ca}}\) are also enhanced in both dilated\textsuperscript{3} and fibrillating\textsuperscript{1} human atria. This suggests that the balance between the various signaling pathways that regulate the current is altered, and that this could contribute to the downregulation of \(I_{\text{Ca}}\). Interestingly, \(I_{\text{Ca}}\) modulation by second messengers differs in several respects between atrial and ventricular myocytes, as illustrated by the effects of serotonin\textsuperscript{11} and soluble tyrosine kinase\textsuperscript{12,13} and by the basal production and degradation of cAMP in atrial myocytes.\textsuperscript{14,15} In pathological conditions, abnormal exposure or enhanced sensitivity of the atrial myocardium to various neurohormones or peptides may enhance the activity of some of these regulatory pathways and thereby contribute to \(I_{\text{Ca}}\) downregulation.

Heart failure is associated with a high incidence of AF.\textsuperscript{16} This is probably because during HF the atrial myocardium undergoes marked structural\textsuperscript{17} and functional remodeling, including \(I_{\text{Ca}}\) downregulation,\textsuperscript{4,18} that could compose an arrhythmogenic substrate for AF. In this setting, calcium current modifications may be caused by a variety of factors, such as increased passive stretch or chronic exposure of the atrial myocardium to abnormal levels of neurohormones and peptides. Using a rat model of ischemic cardiopathy complicated by heart failure, we studied the characteristics of \(I_{\text{Ca}}\) in left atrial myocytes and attempted to identify mechanisms that could contribute to its alterations. We obtained electrophysiological and biochemical evidence that the decreased \(I_{\text{Ca}}\) in atrial myocytes during HF is caused by changes to its cAMP-dependent regulation.
Tissue and Biological Parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=25)</th>
<th>CHF (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>576±8</td>
<td>534±6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.50±0.02</td>
<td>2.04±0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>1.90±0.04</td>
<td>2.30±0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.50±0.09</td>
<td>0.80±0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ANP, pmol/mL*</td>
<td>48.18±1.11</td>
<td>144.48±7.09</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CHF indicates congestive heart failure; BW, body weight; HW, heart weight; RV, right ventricle; LV, left ventricle; ANP, atrial natriuretic peptide; and cGMP, cyclic guanosine monophosphate.

Materials and Methods

Experimental Myocardial Infarction and Clinical Data

Myocardial infarction was induced in 10-week-old male Wistar rats by left coronary artery ligation as previously described.19,20 Animals were obtained from Charles River, France. Animal care complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The studies were performed under authorization No. 006235 of the Ministère de l’Agriculture, France. Sham operations were performed on 25 rats. Three months after infarction of ≤30% to 40% of the left ventricle, 30 animals had clinical, biological, and morphological signs of heart failure while all the animals were in sinus rhythm (Table).

Cardiac Myocyte Preparation

Rats were anesthetized with pentobarbital sodium and after heparinization the chest was opened; the left atrium was removed, cut, and washed in Krebs-Ringer solution containing (in mmol/L) NaCl 35, KCl 4.75, KH2PO4 1.19, NaHPO4 16, HEPES 10, glucose 10, NaHCO3 25, saccharose 134, and 2,3-butanedione oxime 30 to prevent tissue injury during cutting (pH 7.4 adjusted with NaOH), gassed with 95% O2/5% CO2, and maintained at 37°C. Myocytes were suspended in bicarbonate-buffered Tyrode solution containing 2 mmol/L Ca2+ and were incubated at 37°C with continuous gassing with 21% O2 to 5% CO2 for at least 1 hour before use.

Current Measurements and Data Analysis

The whole-cell configuration of the patch-clamp technique (amplifier, Axoclamp 200A, Axon Instruments) was used to record Ica. Borosilicate glass pipettes (tip resistance: 1 to 2 MΩ) were filled with a solution containing (in mmol/L) CsCl 130, MgCl2 2, HEPES 10, EGTA 15, glucose 10, and MgATP 3 (pH was adjusted to 7.2 with CsOH). Myocytes were bathed with a solution containing (in mmol/L) NaCl 35, KCl 4.75, KH2PO4 1.19, NaHPO4 16, HEPES 10, glucose 10, NaHCO3 25, saccharose 134, and 2,3-butanedione oxime 30 to prevent tissue injury during cutting (pH 7.4 adjusted with NaOH), gassed with 95% O2/5% CO2, and maintained at 37°C. Myocytes were isolated from the left atrium by using the same enzymatic used for human atrial myocytes.12 Isolated myocytes were suspended in bicarbonate-buffered Tyrode solution containing 2 mmol/L Ca2+ and were incubated at 37°C with continuous gassing with 21% O2/5% CO2 for at least 1 hour before use.

The amplitude of Ica was calculated as the difference between the peak inward current and the current measured at the end of the test pulse, and its density was obtained by dividing Ica amplitude by the membrane capacitance. The rate of inactivation of Ica was best fitted by the sum of two exponential components: I = Ica,inact[exp(−τ1)] + Ica,act[exp(−τ2)] (Equation 1), where Ica,inact and Ica,act represent the amplitudes, and τ1 and τ2 represent the time constant of the fast and slow component of Ica decay, respectively. The sum of Ica,inact and Ica,act was always equal or less than Ica. For calcium current inactivation measurement, test pulses for Ica were preceded by 2-second conditioning pulses. Activation plots were generated by dividing peak Ica measured at a given potential by the difference between measured and reversal potential. Data on the conductance/voltage activation and inactivation curves were best fitted with a Boltzmann distribution equation: G/Ica = Gmax/[1 + exp(V1/2 - V)] (Equation 2) and δIca = I1/2[1 + exp(V1/2 - V)] (Equation 3), respectively, where G represents the conductance calculated at membrane potential V, I the amplitude of Ica at the conditioning potential V, V1/2 the potential at which half of the channels are activated or inactivated, and k the slope factor. Concentration-response curves were fitted as follows: E = Emax[D/(D + EC50)] (Equation 4), where E is the percentage change in Ica, Emax is the maximal response induced by the drug, and D is the concentration of isoprotroenol tested.

Western Blot

The immunoblotting procedure used to study α1C-subunit expression was that described by Gao et al.21 Frozen atrial tissues were crushed and homogenized on ice in buffer A (250 mmol/L sucrose, 250 mmol/L KCl, 10 mmol/L imidazole [pH 7.4], 5 mmol/L MgCl2, 10 mmol/L EDTA, and protease inhibitors). The homogenates were centrifuged for 10 minutes at 5000g to eliminate debris and nuclei. The pellets were washed with buffer A containing 0.6 mol/L KCl to extract myosin. The pellets were then washed with buffer B (50 mmol/L Tris-HCl [pH 7.4], 2 mmol/L EDTA, 2 mmol/L EGTA, and protease inhibitors) and resuspended in buffer B containing 1% SDS. The amount of protein was determined by the Bradford assay. Protein samples (30 μg per lane) were separated on 10% polyacrylamide-SDS gels and transferred to nitrocellulose membranes for Western blotting with an antibody directed against the α1C-subunit of the Ca2+ channels (1/1000; Alomone Labs). Proteins were detected with enhanced chemiluminescence and horseradish peroxidase. The filters were visualized using the Renaissance kit (NEN Life). To control for the quantity and quality of protein transferred to the membrane, a Coomassie blue staining of total protein was performed and analyzed by densitometric scanning with NIH image software. Moreover, the enhanced chemiluminescence spot was normalized by the integrated density of Coomassie blue–stained proteins of the lane used as an internal standard. Comparison of samples from different blots was made by loading a given sample on the different blots that was used then as a standard to normalize the other density values.22

Atrial Natriuretic Peptide (ANP) and Cyclic Nucleotides Assay

Plasma concentrations of ANP and urinary and extruded cGMP were measured as described previously.19,20,23 Intracellular cAMP was measured with a radioimmunosay kit (Biotrak, Amersham Pharmacia Biotech). Myocytes were washed twice with PBS 1×, and cells were scraped in 250 μL of 0.01 N HCl and frozen in liquid nitrogen until use. Cell extracts were then thawed and sonicated. The lysates were separated by centrifugation (10 000g, 10 minutes), and cAMP was measured in the supernatant. Concentrations of cAMP were normalized to the total content of protein (g · L−1).

Reagents

ANP (rat synthetic) was dissolved in 1% acetic acid, 3-isobutyl-1-methyl-xanthine (IBMX) was dissolved in distilled water, and okadaic acid (OA) was dissolved in ethanol. All drugs were obtained from Sigma Chemical Co, except for isoproterenol (Sanoﬁ Winthrop).

Statistical Analysis

Values are expressed as mean±SEM. Student’s paired t test was used to determine the statistical significance of differences between means. One-way analysis of variance (ANOVA) was used to determine the statistical significance of differences between means in different experimental conditions. Statistical significance was set at P<0.05.
Results

I\textsubscript{Ca} Is Reduced in Atrial Myocytes From Rats With Heart Failure

Figures 1A and 1B show examples of \(I\textsubscript{Ca}\) elicited by incremental (10-mV) 350-ms test pulses from \(-60\) mV recorded in atrial myocytes from sham-operated (S) and heart failure (HF) rats. The activation threshold, the potential of the peak current, and the apparent reversal potential of \(I\textsubscript{Ca}\) were similar in the two groups of myocytes (Figure 1C), but current density was markedly reduced in myocytes from HF (6 rats) compared with S (6 rats) at all potentials at which \(I\textsubscript{Ca}\) activated (at \(10\) mV: \(1.7 \pm 0.1\) pA/pF versus \(4.1 \pm 0.2\) pA/pF, in HF \(n=25\) myocytes versus S \(n=21\) myocytes) rats; \(P<0.001\)). In both sham and HF myocytes, the decay of \(I\textsubscript{Ca}\) was best fitted with a biexponential function (Equation 1) defining an initial fast-inactivating and a second slowly inactivating components. The rate of the fast-inactivating component was decreased in HF (3 rats) compared with S (3 rats) (19.2 \(\pm 1.4\) ms versus \(10.7 \pm 1.1\) ms, \(n=10\), \(P<0.01\) in HF and S, respectively). The steady-state inactivation (Equation 2) and voltage-dependent activation (Equation 3) of \(I\textsubscript{Ca}\) were similar in the two groups of cells (no change in \(V\text{\textsubscript{1/2}}\) and \(k\)) (Figure 1D). Cell size, evaluated by measuring membrane capacitance, was increased in myocytes from HF animals (14 rats), with an apparent bimodal distribution (Figure 2A). However, the reduced amplitude of \(I\textsubscript{Ca}\) was observed independently of cell size (Figure 2B). To identify a possible corresponding decrease in the expression of the L-type Ca\textsuperscript{2+} channel \(\alpha\textsubscript{1C}\)-subunit protein, Western blot analysis was performed on membrane proteins extracted from left atria. As illustrated in Figure 3A, there was no evidence of abnormal degradation of protein extracted from atria of HF rats and stained with Coomassie blue. In both groups, a prominent band at \(\approx200\)-kDa was detected (Figure 3B). Quantitative analysis of the density of the 200-kDa bands revealed a nonsignificant (NS) decrease in protein levels in HF compared with S (3.3 \(\pm 0.2\) versus 2.9 \(\pm 0.2\) [arbitrary units] in S [\(n=12\)] and HF [\(n=19\)] rats, NS, respectively; Figure 3C). Figure 3D shows that the integrated density of the enhanced chemiluminescence spot obtained with the anti-\(\alpha\textsubscript{1C}\)-subunit had a linear dependence on the amount of protein loaded.

Effects of \(\beta\)-Adrenergic Stimulation on \(I\textsubscript{Ca}\) Are Enhanced in Rats With Heart Failure

To determine if the reduced \(I\textsubscript{Ca}\) in HF myocytes was caused by changes in current regulation by second messengers, we tested the effects of the \(\beta\)-adrenergic agent isoproterenol (ISO). As illustrated in Figures 4A and 4B, 10 \(\mu\)mol/L ISO stimulated \(I\textsubscript{Ca}\) in myocytes from both S (3 rats) and HF (5 rats). However, the magnitude of the effects of ISO on \(I\textsubscript{Ca}\) was far greater in HF (2.5-fold, \(n=9\), \(P<0.001\)) than S (1-fold,

---

**Figure 1.** Characteristics of \(I\textsubscript{Ca}\) in atrial myocytes from sham-operated and HF rats. Traces of \(I\textsubscript{Ca}\) elicited by 10-mV incremental 750-ms test pulses from a holding potential of \(-60\) mV and recorded in atrial myocytes from sham-operated (A) and HF (B) rats. C, Current-voltage relationship of \(I\textsubscript{Ca}\) (expressed in current density) in sham (○) and HF (●) rats. Each point is the average of 8 cells. D. Activation and steady-state inactivation of \(I\textsubscript{Ca}\) in sham-operated (○) and HF (●) rats. *\(P<0.05\), †\(P<0.01\), and ‡\(P<0.001\).

**Figure 2.** Characterization of cellular remodeling and the cell-size/current-amplitude relation. A, Distribution of membrane capacitance of atrial myocytes from sham-operated (○) and HF (●) rats. B, Peak current density of each myocyte as a function of its membrane capacitance (cell size) in sham (○) and HF (●) rats.

---
Moreover, following ISO treatment, the difference in $I_{Ca}$ density between myocytes from HF (at 0 mV: 6.1 ± 0.4 pA/pF, n = 9, NS) and S (at 0 mV: 7.3 ± 0.8 pA/pF, n = 10) was no longer significantly different (Figure 4D). Figure 4C shows the concentration-dependent effect of ISO on $I_{Ca}$ recorded in S (11 rats) and HF (12 rats). A greater effect of ISO on $I_{Ca}$ in myocytes from HF compared with S was observed at all concentrations tested whereas the EC$_{50}$ of the agonistic effects was similar in both groups of myocytes: EC$_{50}$ = 1.4 μmol/L (Equation 4). To directly activate the cAMP-dependent protein kinase (PKA), myocytes were dialyzed with an internal solution containing 100 μmol/L cAMP, which caused a marked increase in $I_{Ca}$ in both HF (3 rats) and S (3 rats) myocytes. At steady state, $I_{Ca}$ density was not significantly different between the two groups of myocytes (13.8 ± 1.3 pA/pF, n = 12 in HF versus 14.0 ± 1.4 pA/pF, n = 6, in S, Figure 4D). Pretreating myocytes with 1 μmol/L of the phosphatase inhibitor (OA) caused a prominent increase in $I_{Ca}$ in atrial myocytes from both HF (4 rats) and S (3 rats), again resulting in the suppression of the difference in the current density between the two groups of myocytes (7.1 ± 1.3 pA/pF, n = 8 versus 7.6 ± 1.1, n = 7, Figure 4D). Finally, we measured the cellular cAMP content, which was reduced in atrial myocytes from HF (n = 3 rats) (93 ± 7 fmol·g$^{-1}$·L$^{-1}$, n = 12) compared with S (n = 4 rats) (125 ± 12 fmol·g$^{-1}$·L$^{-1}$, n = 23, P < 0.05).

Enhanced Activity of cGMP-Dependent Signaling Pathways in HF Myocytes

We then examined whether the reduced $I_{Ca}$ in HF atrial myocytes was because of a decreased cellular cAMP concentration caused, for instance, by its increased degradation by...
cGMP-dependent phosphodiesterases (PDEs). We first studied the effects on $I_{Ca}$ of the phosphodiesterase inhibitor IBMX. IBMX at 10 μmol/L increased $I_{Ca}$ in myocytes from both S (3 rats) (4.5±0.8 pA/pF in control versus 5.2±1.0 pA/pF in IBMX-treated conditions, n=9, NS) and HF (4 rats) (1.4±0.1 pA/pF in control versus 2.5±0.3 pA/pF in IBMX-treated conditions, n=8, P<0.001). However, the effect of IBMX on $I_{Ca}$ was significantly stronger on myocytes from HF (76.0±11.2%, n=8, P<0.05) relative to S (15.8±21.2%, n=9) (only 45% of S myocytes reacted to IBMX) (Figure 5A). In another attempt to characterize the GMP-dependent regulation of $I_{Ca}$, we tested the effects of ANP, which causes intracellular accumulation of cGMP. ANP at 10 nmol/L decreased $I_{Ca}$ in both S (3 rats) (3.6±0.3 pA/pF in control versus 1.5±0.1 pA/pF in ANP-treated conditions, n=6, P<0.01) and HF (3 rats) (1.9±0.4 pA/pF in control versus 1.3±0.1 pA/pF in ANP-treated conditions, n=6, NS) atrial myocytes, but the effect was more pronounced in myocytes from HF (54.1±4.8%) than in HF myocytes (24.3±8.8%, P<0.001) (Figure 5B). $I_{Ca}$ inhibition by ANP was suppressed in both groups of cells by pretreatment with 100 μmol/L IBMX (not shown). The cGMP concentration was measured 2 hours after myocyte isolation in the medium bathing the cells. As cGMP is not degraded in the extracellular medium, its concentration is a reliable indication of its intracellular level. There was a marked increased in cGMP concentrations in myocyte preparations from HF compared with S (55.8 177±8.0 pmol · mL⁻¹ versus 6.2 177±4.0 pmol · mL⁻¹ in HF [n=4] and S [n=4] rats, respectively; P<0.05) (Figure 5C).

Discussion

This study shows that the density of the L-type Ca²⁺ current recorded in atrial myocytes isolated from the left atrium of rats with heart failure is lower than in those isolated from healthy animals. This downregulation of $I_{Ca}$ appears, at least in part, to be because of changes in its regulation by intracellular second messengers.

The size of atrial myocytes from HF animals was increased, indicating a degree of hypertrophy. However, this could not account for the reduced $I_{Ca}$ in HF because the latter was independent of cell size, as during atrial fibrillation in humans. Biochemical studies of right atrial tissue from patients with AF show that a significant decrease in levels of mRNA coding for L-type Ca²⁺ channels is detected after more than 3 months of sustained AF. The latter findings are corroborated by the nearly 2-fold decrease in the protein level of the calcium channel α₁C-subunit in chronically fibrillating atria only. In the present study, α₁C-subunit expression was only slightly decreased in the atrial myocardium of rats with HF. Apart from species differences, the discrepancy between our results and those of the literature may be because prolonged, sustained AF in humans and HF lasting 2 to 3 months in rats does not cause the same phenotype alterations. In dogs, rapid atrial activation results in a decrease in channel expression and the maximum level of dihydropyridine binding, pointing to a possible specific role of the high beating rate in the regulation of channel expression. Moreover, a recent study shows that the level of calcium channel is not altered during mitral valve diseases, which are usually complicated by hemodynamic overload of the atria and $I_{Ca}$ downregulation. Of note, most published studies show a marked scattering of calcium channel expression values, with an overlap between control and AF specimens, suggesting that the reduction in channel expression may not be present in all patients. Interestingly, during tachycardia-induced AF in dogs, the discrepancy between the 40% decrease in dihydropyridine receptor levels and the 70% decrease in $I_{Ca}$ has been considered as an indication that factors other than the sole reduction in calcium channel density contribute to the downregulation of $I_{Ca}$.

In a previous study, we found that the small $I_{Ca}$ in myocytes from hemodynamically overloaded and dilated human atria was highly responsive to β-adrenergic agonists: in the presence of ISO, current density was no longer significantly different between myocytes obtained from healthy and diseased atria. Enhanced effect of β₁-adrenergic regulation on $I_{Ca}$ was also observed in myocytes isolated from left and right atria of patients with chronic AF. These results point to the existence of alterations of the regulation of $I_{Ca}$ by second messengers in diseased atria. In cardiac myocytes, calcium current is regulated by β-adrenergic agonists through a cAMP-dependent signaling pathway. Single-channel studies have shown that PKA increases the availability and opening probability of the calcium channel α₁C-subunit. In addition, it has been suggested that Ca²⁺ channels in cardiac myocytes are phosphorylated in the absence of any neurohormonal stimulation of the cell and that this basal phosphorylation is necessary to maintain normal channel function. This is illustrated by the observation that the rundown of Ca²⁺ channels following membrane patch excision is reversed by...
application of MgATP and PKA at the inside face of the membrane. The present finding that interventions known to stimulate cAMP-dependent phosphorylation directly (β-adrenergic cell exposure, intracellular cAMP application) or indirectly (phosphatase inhibition) restore ICa in HF myocytes indicates that reduced basal cAMP-dependent regulation of ICa could be an important mechanism underlying its decrease during HF. The high value of the EC50 of the ISO effect on ICa compared with published data might reflect tissue specificity of, for instance, the density of the β-adrenergic receptors as reported in neonatal dog heart. The restoration of ICa density by cAMP-dependent mechanisms may be caused by an excessive cAMP degradation by cGMP-dependent phosphodiesterases, as suggested by the increased cellular cGMP content and the high sensitivity of ICa to IBMX (whereas ICa was poorly sensitive to ANP in HF myocytes). ANP, whose plasma concentration is enhanced in HF as in our model, stimulates the cGMP accumulation in atrial myocytes and may be an important factor for the decreased cAMP-dependent regulation of the basal current in this setting. However, in the presence of IBMX, ICa remained smaller in HF than in control myocytes. Besides incomplete blockade of PDE by IBMX, or a nonspecific effect of the compound on ICa, this observation suggests that basal cAMP production may be lower in the former than in the latter, possibly because of increased G1 protein activity in HF. Mechanisms other than PDE, such as the enhanced phosphatase activity, can also contribute to the low basal cAMP-dependent regulation of ICa. Indeed, as in other species and myocyte types, the ICa of rat atrial myocytes is highly sensitive to phosphatase inhibition; these enzymes appear to play an important role in the basal regulation of the current, keeping with the tight coupling between Ca2+ channels and OA-sensitive phosphatases. Various studies have shown that the kinetics of ICa inactivation also depend on cAMP-dependent regulation of the current and the slowly decaying ICa in HF may be another consequence of its downregulation. However, other mechanisms may explain the slow rate of ICa inactivation in atrial myocytes from HF. For instance, in overloaded atria as during AF, the sarcoplasmic reticulum function may be altered so that less Ca2+ accumulates at the cytoplasmic site of channels, causing a reduction in the rate of Ca2+-dependent inactivation of ICa.

Heart failure is generally associated with downregulation of the β-adrenergic response of ventricular myocytes, in contrast to the enhanced response of atrial myocytes observed here. The mechanisms underlying the inefficient adrenergic stimulation in failing ventricular myocardium are complex and include β-receptor downregulation, increased G1-protein activity, and decreased Gβ-protein activity. These mechanisms may not operate in the atrial myocardium, or alternatively, the interval between myocyte isolation and current recording may permit the restitution of possibly internalized receptors. For instance, the ICa of ventricular myocytes from Syrian hamsters (Bio 14.6) with heart failure responds normally to β-adrenergic stimulation. Of note, adenylyl cyclase activity is normal in atrial myocytes isolated from the right appendage of patients with heart failure, suggesting that the β-adrenergic pathway is functional.

Study Limitations and Potential Implications
There are two important limitations in our study: the relatively low temperature used for our experiments; and the fact that our data were obtained ex vivo, ie, in the absence of neurohormonal stimulation. In vivo, cardiac function is permanently subjected to complex regulatory effects exerted by a number of peptides that could be enhanced during heart failure and modulate ICa. Thus, calcium current regulation by second messengers and, in turn, its amplitude, must be highly variable, making it difficult to determine the precise contribution of the apparent decrease in the number of active calcium channels observed here to the electrical remodeling of the atrial myocardium. We can only speculate that normal ICa activation may be more dependent on adrenergic stimulation in diseased than in healthy atrial myocardium. A number of studies using multicellular preparations at physiological temperature have shown that catecholamines restore the depressed electrical activity properties of hemodynamically overloaded and fibrillating atria both in humans and in animal models. For instance, in dogs with mitral valve fibrosis, nonexcitable myocytes recover a normal action potential on norepinephrine application. It is also possible that the increased effects of catecholamines on ICa are involved in the adrenergic dependency of atrial arrhythmia. Further studies conducted in situ are necessary to examine the role of calcium current modulation by second messengers in the occurrence and perpetuation of AF.

Acknowledgments
This work was supported by grants from the Association Française contre les Myopathies and Fondation de France. Christophe Boixel was supported by a grant from Ministère de l’Enseignement Supérieur et de la Recherche.

References
34. Davare MA, Horne MC, Hell JW. Protein phosphatase 2A is associated with class C L-type calcium channels (Ca2, 1,2) and antagonizes channel phosphorylation by cAMP-dependent protein kinase. *J Biol Chem.* 2000;275:39710–39717.
Mechanisms of L-Type Ca\textsuperscript{2+} Current Downregulation in Rat Atrial Myocytes During Heart Failure

Christophe Boixel, Walter Gonzalez, Liliane Louedec and Stéphane N. Hatem

Circ Res. 2001;89:607-613; originally published online September 13, 2001;
doi: 10.1161/hh1901.096702

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/89/7/607

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/