Changes in Ca$^{2+}$ Cycling Proteins Underlie Cardiac Action Potential Prolongation in a Pressure-Overloaded Guinea Pig Model With Cardiac Hypertrophy and Failure

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Abstract—Ventricular arrhythmias are common in both cardiac hypertrophy and failure; cardiac failure in particular is associated with a significant increase in the risk of sudden cardiac death. We studied the electrophysiologic changes in a guinea pig model with aortic banding resulting in cardiac hypertrophy at 4 weeks and progressing to cardiac failure at 8 weeks using whole-cell patch-clamp and biochemical techniques. Action potential durations (APDs) were significantly prolonged in banded animals at 4 and 8 weeks compared with age-matched sham-operated animals. APDs at 50% and 90% repolarization (APD$_{50}$ and APD$_{90}$ in ms) were the following: 4 week, banded, 208±51 and 248±49 (n=15); 4 week, sham, 189±68 and 213±69 (n=16); 8 week, banded, 197±40 and 226±40 (n=21); and 8 week, sham, 156±42 and 189±45 (n=22), respectively; $P<0.05$ comparing banded versus sham-operated animals. We observed no significant differences in the K$^+$ currents between the 2 groups of animals at 4 and 8 weeks. However, banded animals exhibited a significant increase in Na$^+$ and Na$^+$-Ca$^{2+}$ exchange current densities compared with controls. Furthermore, we have found a significant attenuation in the Ca$^{2+}$-dependent inactivation of the L-type Ca$^{2+}$ current in the banded compared with sham-operated animals, likely as a result of the significant downregulation of the sarcoplasmic reticulum Ca$^{2+}$ ATPase, which has been documented previously in the heart failure animals. Our data provide an alternate mechanism for APD prolongation in cardiac hypertrophy and failure and support the notion that there is close interaction between Ca$^{2+}$ handling and action potential profile. (Circ Res. 2000;86:558-570.)

Key Words: cardiac hypertrophy • cardiac failure • action potential • guinea pig • Ca$^{2+}$ current

E ven though ventricular arrhythmias are common in both cardiac hypertrophy and failure,1,2 cardiac failure in particular is associated with a significant increase in the risk of sudden cardiac death.3 Indeed, sudden cardiac death is one of the most common causes of death in heart failure patients, occurring with roughly the same frequency as pump failure. The underlying basis for this increase in arrhythmias is not known. Numerous studies have provided evidence using a variety of animal models for cardiac hypertrophy and failure, as well as tissues from human heart failure, that multiple electrophysiological and biochemical alterations occur during hypertrophy and heart failure. Most studies have identified a prolongation in cardiac action potentials.4–9 These changes in the repolarization have been found to be associated with reduction of transient outward K$^+$ current (I_o) and inward rectifier K$^+$ current (I_K1).9,10 There are alterations in sarcoplasmic reticulum (SR) Ca$^{2+}$-handling proteins.11–15 Finally, the plasma membrane Na$^+$-Ca$^{2+}$ exchanger is upregulated in some heart failure models.16,17

Previous studies of heart failure have shown a downregulation of I_o as one of the major causes of action potential prolongation. However, this current (I_o) is absent in guinea pig ventricular myocytes. Nonetheless, there is marked prolongation of action potential duration (APD) in a guinea pig model with cardiac hypertrophy and failure, similar to that in human, rat, dog, and cat, where I_o is prominent.18 Thus, the guinea pig model may provide an additional alternative mechanism for action potential alteration in cardiac hypertrophy and failure.

We investigated the electrophysiologic and biochemical changes in a guinea pig model with cardiac hypertrophy, which reliably progresses to cardiac failure with a predictable time course. In this model, thoracic aortic banding of guinea pigs for 4 weeks produces compensated left ventricular (LV) hypertrophy, which is defined as increased LV mass-to–body mass ratio, normal LV contractile function, and no pulmonary congestion as indexed by the lung-to–body weight ratio. However, after aortic banding for 8 weeks, the decompen-
sated phenotype appears as manifested by LV hypertrophy, contractile depression, and pulmonary congestion. Thus, this guinea pig model of thoracic aortic banding provides a continuum from normal to compensated and decompensated hypertrophy with congestive heart failure.

We have identified at least 4 biophysical and biochemical changes associated with cardiac hypertrophy and failure in the guinea pig model, as follows. (1) APDs are significantly prolonged during both cardiac hypertrophy and failure as compared with those of the age-matched controls. (2) These changes in the repolarization process are not associated with changes in the K$^+$ current density. (3) There is a significant upregulation of the Na$^+$ current density and Na$^+$/Ca$^{2+}$ exchanger current density. (4) Finally, whereas there are no changes in the Ca$^{2+}$ current density, the Ca$^{2+}$-dependent inactivation of the L-type Ca$^{2+}$ channel is significantly attenuated in cardiac hypertrophy and failure. The decrease in the Ca$^{2+}$-dependent inactivation of the Ca$^{2+}$ current likely stems from the downregulation of the SR Ca$^{2+}$ ATPase, coupled with the upregulation of the Na$^+$/Ca$^{2+}$ exchanger proteins, leading to a decrease in the SR Ca$^{2+}$ load and Ca$^{2+}$-induced Ca$^{2+}$ release. These changes in the kinetics of the L-type Ca$^{2+}$ channel may underlie, at least in part, the lengthening of the APD during cardiac hypertrophy and failure.

**Materials and Methods**

**Compensated Cardiac Hypertrophy and Failure Model in Guinea Pigs**

LV hypertrophy and failure were induced in adult male Charles River guinea pigs (250 to 300 g) by chronic pressure overload using subtot al descending thoracic aortic banding as described. Sham-operated animals underwent the same operation, except that the aorta was not banded.

**Electrophysiological Recordings**

Single LV myocytes were isolated as previously described. Action potentials were recorded using the perforated patch technique. All other experiments were performed using the conventional whole-cell patch-clamp technique. Action potentials and delayed rectifier K$^+$ currents were recorded at 36±0.5°C, whereas other currents were recorded at room temperature.

**Solutions**

For action potential recordings, the patch pipettes were backfilled with amphotericin (200 μg/ml). Pipette solution contained (in mmol/L) potassium glutamate 120, KCl 25, MgCl$_2$ 1, and HEPES 10 (pH 7.4 with KOH). The external solution contained (in mmol/L) NaCl 138, KCl 4, MgCl$_2$ 1, CaCl$_2$ 2, NaH$_2$PO$_4$ 0.33, glucose 10, and HEPES 10 (pH 7.4 with NaOH). For K$^+$ current recordings, the external solution contained (in mmol/L) NaCl 132, KCl 4, CaCl$_2$ 1.8, MgCl$_2$ 1.2, glucose 5, and HEPES 10 (pH 7.4 with NaOH), and pipette solution contained (in mmol/L) KCl 140, Mg-ATP 4, MgCl$_2$ 1, EGTA 5, and HEPES 10 (pH 7.3 with KOH). Nimbopine (0.01 mmol/L) was added to the external solution to block the L-type Ca$^{2+}$ current. Na$^+$ and T-type Ca$^{2+}$ currents were inactivated by a holding potential of −40 mV. For whole-cell Na$^+$ currents, the external solution contained NaCl 5, CsCl 5, tetraethyl ammonium (TEA) chloride 130, MgCl$_2$ 1.8, glucose 10, and HEPES 10 (pH 7.4 with TEA-OH), and the pipette solution contained (in mmol/L) NaCl 15, CsF 150, EGTA 10, and HEPES 10 (pH 7.2 with CsOH). For whole-cell Ca$^{2+}$ currents, the external solution contained (in mmol/L) N-methyl-d-glucamine 140, CsCl 5, CaCl$_2$ 2, MgCl$_2$ 0.5, glucose 10, and HEPES 10 (pH 7.4 with HCl), and the pipette solution contained (in mmol/L) CsCl 125, TEA-Cl 20, EGTA 10 or BAPTA (Molecular Probes) 0.05, Mg-ATP 4, and HEPES 10 (pH 7.3 with CsOH). Na$^+$/Ca$^{2+}$ exchanger currents were recorded as previously described using external solution containing (in mmol/L) NaCl 135, CaCl$_2$ 2, CsCl 10, MgCl$_2$ 1, TEA-Cl 10, glucose 10, HEPES 10, rymodine 0.01, nifedipine 0.01, and tetrodotoxin 0.02 (pH 7.3 with NaOH) and pipette solution containing (in mmol/L) CsCl 135, MgCl$_2$ 2, NaCl 15, EGTA 0.2, and HEPES 10 (pH 7.2 with CsOH).

**Western Blot Analysis**

The relative protein levels of Na$^+$/Ca$^{2+}$ exchanger were determined from LV homogenates obtained from aortic-banded and sham-operated animals at 4 and 8 weeks using quantitative immunoblot techniques. Equal amounts of protein extract from the 4 groups of animals (50 μg/lane) were subjected to electrophoresis as described. The separated proteins were transferred to nitrocellulose membranes. Blots were incubated with a polyclonal antibody for Na$^+$/Ca$^{2+}$ exchanger (Swant) and in sequential steps with peroxidase-conjugated secondary antibody (goat anti-rabbit IgG, Kirkegaard & Perry Laboratories) and detected by the enhanced chemiluminescent detection system (Amersham Life Science). Relative protein level was determined by normalization to the level of actin, which was used as the internal control.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

**Results**

**Validity of the Guinea Pig Model**

Figure 1A demonstrates that thoracic aortic banding of guinea pigs for 4 weeks produces a significant increase in LV mass-to-body mass ratio without evidence of pulmonary congestion, as indexed by the lung-to-body weight ratio. In contrast, after aortic banding for 8 weeks, the decompensated phenotype appears as manifest by LV hypertrophy and pulmonary congestion (Figure 1A). The documented increase in LV mass at 4 and 8 weeks was found to be associated with cellular hypertrophy. Cell capacitance of 4- and 8-week aortic-banded animals was significantly increased as compared with that of cells obtained from the age-matched sham-operated animals. Capacitance (in pF) measured from cells isolated from 4-week banded versus sham animals were 232±50 (n=40 cells, 25 animals) versus 200±42 (n=53 cells, 26 animals), respectively; P=0.0017. The data obtained for the corresponding 8-week banded and sham animals were 322±69 (n=50 cells, 26 animals) versus 280±59 (n=51 cells, 28 animals), respectively; P=0.001. The cell capacitance was calculated by integrating the area under an uncompensated capacitive transient elicited by a 20-mV hyperpolarizing pulse from a holding potential of −40 mV.

The deterioration in the contractile function in aortic-banded guinea pigs has previously been described in this model. At 4 weeks after aortic banding, there is preservation of LV contractile function; however, after 8 weeks, there is contractile depression associated with pulmonary congestion. Thus, this guinea pig model of thoracic aortic banding provides a continuum from normal to compensated and then to decompensated hypertrophy with congestive heart failure.

**Duration of Action Potentials Is Markedly Prolonged in Cardiac Hypertrophy and Failure Myocytes, Which Do Not Express I$_{to}$**

Guinea pig ventricular myocytes do not express the 4-amino pyridine (4-AP)-sensitive transient outward K$^+$ current.
The action potential profile was unchanged after application of 1 mmol/L 4-AP. In contrast, there is substantial prolongation of the action potentials after application of 100 μmol/L of Ba⁺² (the terminal portion of the action potential) or 10 μmol/L of E4031, each of which blocks the inward rectifier K⁺ current (I⁻Kr) or the rapid component of the delayed rectifier K⁺ current (I⁺Kr), respectively (Figure 1B). Shown in Figure 1C and 1D are action potentials recorded from isolated cardiac myocytes from hypertrophy and failure animals. The APD measured at 50% and 90% repolarization (APD₅₀ and APD₉₀) obtained from the hypertrophy and failure animals were significantly prolonged as compared with the age-matched sham-operated animals. These changes were not associated with changes in the resting membrane potentials. To understand the mechanisms for the observed changes in the APD in cardiac hypertrophy and failure animals, we examined the ionic currents and Na⁺-Ca²⁺ exchange current.

**I⁻Kr and I⁺Kr Remain Unchanged During Cardiac Hypertrophy and Failure**

Guinea pig ventricular myocytes exhibit 2 components of delayed rectifier K⁺ currents, which are the rapidly activating (I⁻Kr) and the slowly activating (I⁺Kr) delayed rectifier K⁺ currents. I⁻Kr can be specifically blocked by the benzenesulfonamide drugs, eg, E4031 or β-sotalol. We measured both components of the K⁺ currents in LV myocytes isolated from banded and sham-operated animals at 4 and 8 weeks after surgery. Depolarizations (2 seconds each) activated an outward current, which grew larger over time (Figure 2A). Repolarizations to −40 mV evoked outward tail currents that decayed gradually. Currents were recorded before and after exposure to 10 μmol/L doxofylline, which blocks the I⁻Kr component. The drug-sensitive component (difference current, I⁻Kr) was obtained by subtraction of the currents recorded in the presence of doxofylline from control records. The drug-sensitive component demonstrates an activation threshold, which is more negative than the drug-insensitive component (I⁺Kr). I⁺Kr does not exhibit any obvious delay in onset and is fully activated during the pulse. In contrast, I⁻Kr is time dependent and shows a more depolarized activation threshold. Figure 2B compares the peak tail currents obtained at −40 mV after depolarization to various potentials of the drug-sensitive (I⁻Kr) and -insensitive (I⁺Kr) components. Another feature that is characteristic of the I⁻Kr is evident; the tail current magnitude saturates with increased depolarization (Figure 2B).

**Figure 1.** A, Effects of aortic banding on heart, lung, and liver weight. Data are shown as mean ± SEM of heart, lung, and liver weight (in g) per body weight (in kg). At 4 weeks, banded animals showed a significant increase in only heart/body weight ratio (n=25 and 26 sham and banded animals at 4 weeks, respectively). In contrast, at 8 weeks, there were significant increases in both the heart/body weight and lung/body weight ratios (n=26 and 28 for sham and banded animals at 8 weeks). B, Different underlying repolarizing currents in guinea pig cardiac myocytes. Action potentials recorded from 3 different control cells at 37°C at a stimulation frequency of 0.5 Hz at baseline (solid lines) and after application of 1 mmol/L 4-AP, 100 μmol/L Ba²⁺, or 10 μmol/L E4031 (dotted lines). C, Differences in the action potential shape and duration in sham-operated and aortic-banded animals at 8 weeks. Representative action potentials were recorded at 37°C in single ventricular myocytes isolated from control (left panel) and failing (right panel) hearts at a stimulation frequency of 0.5 Hz. Action potentials recorded from myocytes isolated from failing ventricles were significantly prolonged compared with control myocytes. The resting membrane potential was unchanged. At 4 weeks, there was also a significant prolongation of the action potentials in myocytes isolated from hypertrophy hearts as compared with the control at the same time point. D, Summary data comparing the APD₅₀ and APD₉₀ between the sham-operated and banded animals at 2 different time points (4 and 8 weeks). Action potentials were recorded at 37°C at a stimulation frequency of 0.5 Hz. Data are mean ± SEM. n=8 to 12 cells from each group. *P<0.05 comparing sham and banded cells at 4 and 8 weeks.
Data were obtained from 4 different groups of animals, sham-operated and banded animals at 4 and 8 weeks each. We compared the peak $I_{K1}$ and $I_{Ks}$ current density between the sham and banded animals at 2 different time points. $I_{K1}$ and $I_{Ks}$ tail current density measured at -40 mV after depolarization to +40 mV (in pA/pF) for sham versus banded animals at 4 and 8 weeks were as follows: $I_{K1}$, 0.43±0.14 (n=7) versus 0.51±0.13 (n=8, $P=NS$), and 0.52±0.09 (n=14) versus 0.63±0.11 (n=15, $P=NS$); $I_{Ks}$, 0.49±0.13 (n=7) versus 0.48±0.04 (n=8, $P=NS$), and 0.45±0.08 (n=14) versus 0.44±0.06 (n=15, $P=NS$).

$I_{K1}$ Is Unchanged in Cardiac Hypertrophy and Failure

The inward rectifier K$^+$ current ($I_{K1}$) is important in maintaining the resting membrane potential and the terminal repolarization phase of the action potential (Figure 1). $I_{K1}$ current density was measured from the cardiac hypertrophy and failure animals and compared with the age-matched sham-operated controls (Figure 3). There were no significant differences in the current density measured from banded animals as compared with sham-operated animals at both 4 and 8 weeks. For example, peak $I_{K1}$ densities (in pA/pF) elicited from a holding potential of -40 to -100 mV were -8.0±0.6 (n=7), -11.3±0.7 (n=8), -9.3±0.1 (n=9), and -11.0±0.6 (n=5) for sham versus banded at 4 and 8 weeks, respectively ($P=NS$).

Na$^+$ Current Density Was Significantly Increased in Cardiac Hypertrophy and Failure

Because the shape and duration of action potentials are critically dependent on the underlying inward and outward currents, we next compared the current density of the inward Na$^+$ and Ca$^{2+}$ currents between the banded and the sham-operated animals. Na$^+$ currents were carefully examined among the 4 groups of animals under symmetrical recording conditions to reduce the magnitude of the current to ensure adequate voltage control. Currents were activated from a holding potential of -40 mV. Current density was obtained by normalizing the measured current to cell capacitance. The 2 different components of the delayed rectifier K$^+$ currents ($I_{K1}$ and $I_{Ks}$) were compared between the sham-operated and banded animals at 2 different time points (cells, n=7, 8, 14, and 15; animals, n=5, 4, 7, and 7 for sham and banded animals at 4 and 8 weeks, respectively). Data are mean±SEM.
peaked near 2 V sham-operated and banded animals at the 2 different time points (4 and 8 weeks). The relations were well described by a Boltzmann function. There were no significant changes in the half-threshold for activation was similar between the banded and sham-operated animals. The inactivation voltages and the maximum slope factors between the groups (Figure 4E and 4F; $V_{1/2}$, $-76.2\pm0.07$, $-76.0\pm0.06$, $-75.7\pm0.09$, and $-74.8\pm0.06$ mV; maximum slope factors, $5.9\pm0.06$, $6.1\pm0.05$, $5.8\pm0.05$, and $5.7\pm0.08$ mV for sham-operated and banded animals at 4 and 8 weeks, respectively; n=7 for each group, $P=NS$).

To determine whether the observed changes in Na$^+$ current density in cardiac hypertrophy and failure are associated with altered kinetics, the time course of inactivation was assessed by biexponential fits to the whole-cell current decay over a range of test potentials from $-50$ to $-15$ mV. Figure 5A shows examples of the biexponential fits with time constants indicated below the current traces. The increase in the Na$^+$ current densities in cardiac hypertrophy and failure was not associated with changes in the time course of inactivation (Figure 5B). The time constants of current decay in cells from cardiac hypertrophy and failure animals and the age-matched sham-operated animals overlapped at all potentials. The recovery kinetics were determined at $-80$, $-100$, and $-120$ mV using standard 2-pulse protocols (Figure 5C through 5E). Repriming of the Na$^+$ current in cells isolated from cardiac hypertrophy and failure hearts and control cells was well fitted with single exponential functions. The time constants of the recovery kinetics at all 3 different potentials did not differ between the groups.

### Current Densities of L-Type and T-Type Ca$^{2+}$ Current Were Unaltered

The 2 different populations of Ca$^{2+}$ currents were separated by differences in their steady-state inactivation (Figure 6). Currents were activated from holding potentials of $-90$ and $-50$ mV using a stimulation frequency of 0.1 Hz. Depolarization from a holding potential of $-50$ mV inactivates a small portion of the current, which has a rapid activation and inactivation kinetics, as expected for the T-type current. The T-type Ca$^{2+}$ currents were then determined by subtraction of traces obtained at a holding potential of $-50$ mV from those obtained at a holding potential of $-90$ mV. There were no significant differences in the L- or T-type current density between the sham-operated or banded animals at the 2 different time points (4 and 8 weeks, Figure 6). However, there was a significant alteration in the inactivation kinetics of the L-type Ca$^{2+}$ channels in the banded animals (Figure 7).

### Ca$^{2+}$-Dependent Inactivation of the L-Type Ca$^{2+}$ Current Was Significantly Attenuated in Cardiac Hypertrophy and Failure

Figure 7 shows representative current traces obtained at a test potential of $+10$ mV from a holding potential of $-55$ mV obtained from banded compared with sham-operated animals using low (50 mmol/L BAPTA) versus high (10 mmol/L EGTA) intracellular Ca$^{2+}$ buffers. In experiments performed with reduced internal Ca$^{2+}$ chelator, 2 mmol/L Ca$^{2+}$ or Ba$^{2+}$ was used as charge carrier, because Ba$^{2+}$ can permeate the Ca$^{2+}$ channel but does not lead to Ca$^{2+}$-induced Ca$^{2+}$ release. In the presence of low internal Ca$^{2+}$ chelator and Ca$^{2+}$ as charge carrier, the inactivation of the L-type Ca$^{2+}$ current was markedly slowed in cells isolated from banded animals as compared with control cells. Currents were well fit using 2 exponential functions with a fast and a slow time constant ($\tau_1$, $\tau_2$).
Figure 4. Na⁺ current density was increased in hypertrophy and heart failure animals. A, Examples of families of whole-cell Na⁺ currents elicited by a series of voltage-clamp steps from a holding potential of −100 mV obtained from sham-operated and banded animals at 2 different time points (4 and 8 weeks, respectively). The cell membrane capacities were 189, 245, 153, and 283 pF, and the effective series resistances (after 90% compensation) were 0.50, 0.28, 0.28, and 0.17 MΩ for the examples shown from sham-operated and banded animals at 4 and 8 weeks, respectively. B, Summary data comparing current density from sham (○, ■) and banded (□, ■) animals. Data are mean±SEM. No. of cells=5, 17, 6, and 7, and No. of animals=3, 4, 3, and 3, for sham-operated and banded animals at 4 and 8 weeks, respectively. Effective series resistances (after 90% compensation) were 0.29±0.05, 0.27±0.02, 0.27±0.03, and 0.30±0.03 MΩ for sham-operated and banded animals at 4 and 8 weeks, respectively. C and D, Voltage dependence of steady-state activation as determined from normalized conductance in banded animals (□, ■) as compared with sham-operated controls (○, ■) at 4 and 8 weeks. Data are mean±SEM. The continuous line fitted to the data points is a Boltzmann function, with half-activation voltages at −47.1±0.2, −46.1±0.4, −47.0±0.2, and −47.7±0.3 mV and maximum slope factors of 4.8±0.2, 5.5±0.3, 4.4±0.2, and 5.1±0.3 mV for sham-operated and banded animals at 4 and 8 weeks, respectively (for each group, No. of cells=4, and No. of animals=3). E and F, Voltage dependence of steady-state inactivation from the 4 different groups of animals. Conditioning pulses were 500 ms in duration at a frequency of 0.1 Hz. A short gap (1 ms) was interposed between the conditioning and test pulses to ensure that the activation variables were constant at the beginning of the test pulse (0 mV). Test current amplitudes were normalized to the value at conditioning potentials of −140 mV. Data are mean±SEM. The continuous line fitted to the data points is a Boltzmann function, with half-inactivation voltages at −76.2±0.1, −76.0±0.1, −75.7±0.1, and −74.8±0.1 mV and maximum slope factors of 5.9±0.1, 6.1±0.1, 5.8±0.1, and 5.7±0.1 mV for sham-operated and banded animals at 4 and 8 weeks, respectively (for each group, No. of cells=8 to 7, and No. of animals=3).
Figure 5. There were no significant differences in the inactivation kinetics of the Na⁺ currents in the 2 different groups of animals at 2 different time points. A, Examples of Na⁺ current traces at step potentials of −40 and −20 mV from a holding potential of −100 mV obtained from the 2 different groups of animals. The current traces were fitted using 2 exponential functions with the fast and slow time constants ($\tau_{\text{fast}}$, $\tau_{\text{slow}}$) as indicated (in ms). B, Summary of the fast and slow Na⁺ current inactivation time constants comparing the 2 different groups of animals at 4 and 8 weeks. Data are mean±SEM. For each group, No. of cells=5 to 8, and No. of animals=3. C and D, Recovery kinetics at −80 mV comparing the 2 different groups of animals at 4 and 8 weeks. Recovery kinetics were determined at −80, −100, and −120 mV using standard 2-pulse protocols. Cells were held at −140 mV. Currents obtained at different recovery intervals were normalized to the maximum currents. Data are mean±SEM. n=6 cells, 3 animals from each group. Data were fitted using a single exponential function (solid lines). Insets show examples of the current traces obtained using the 2-pulse protocols from 2 cells isolated from hypertrophy and heart failure animals, respectively. E, Summary data of time constants of recovery at −80, −100, and −120 mV from the 2 different groups of animals at 4 and 8 weeks. The holding potential used in these experiments was −140 mV at a frequency of stimulation of 0.1 Hz. Data are mean±SEM. For each group, No. of cells=4 to 6, and No. of animals=3.
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and \( \tau_i \) were significantly prolonged in the banded animals, as follows: \( \tau_i \) (in ms) were 16.3±1.1 versus 22.9±2.2 \((P=0.02)\), and \( \tau_t \) were 120.5±8.4 versus 114.4±12.8; \( P=\text{NS} \) for sham \((n=9)\) as compared with banded \((n=11)\) animals at 8 weeks. The differences in the time constant of inactivation between the 2 groups of animals were completely abolished in the presence of high intracellular \( \text{Ca}^{2+} \) buffer (Figure 7B) or when \( \text{Ba}^{2+} \) was used as the charge carrier (Figure 7C). The \( \text{Ba}^{2+} \) currents were well fit using one exponential function (\( \tau \) were \( 137±10.9 \) versus \( 117±5.6 \) ms; \( P=\text{NS} \) comparing sham \([n=9]\) and banded \([n=11]\) animals at 8 weeks). \( \text{Ca}^{2+} \) currents recorded in the presence of 10 mmol/L EGTA were well fit using 2 exponential functions. As expected, both \( \tau_i \) and \( \tau_t \) were prolonged compared with recordings obtained from low \( \text{Ca}^{2+} \) buffering condition. However, there were no significant differences in both \( \tau_i \) and \( \tau_t \), between the control and experimental animals; \( \tau_i \) were \( 37.1±3.2 \) versus \( 44.2±4.9 \), and \( \tau_t \) were \( 140.7±2.6 \) versus \( 165±30.9 \); \( P=\text{NS} \) for sham \((n=9)\), as compared with banded \((n=11)\) animals at 8 weeks. Similar data were obtained at 4 weeks. These findings are consistent with an attenuation of the \( \text{Ca}^{2+} \)-dependent inactivation of the L-type \( \text{Ca}^{2+} \) current in this model of cardiac hypertrophy and failure. This decrease in the \( \text{Ca}^{2+} \)-dependent inactivation is likely due to the decrease in the sarcoplasmic/endoplasmic reticulum \( \text{Ca}^{2+} \) ATPase pumps\(^{13}\) coupled with a compensatory increase in the \( \text{Na}^{+}-\text{Ca}^{2+} \) exchanger (Figure 8) leading to a decrease in the SR \( \text{Ca}^{2+} \) load. Indeed, a depression in the \( \text{Ca}^{2+} \) transients as assessed by a \( \text{Ca}^{2+} \) indicator dye, Fura-2, has previously been documented in this model.\(^{26}\) There was a decrease in the peak systolic \([\text{Ca}^{2+}]_i\), in hypertrophy animals with further depression in hypertrophy animals with congestive heart failure compared with the control animals. This depression was associated with a decrease in the time to peak and relaxation of the \( \text{Ca}^{2+} \) transients.\(^{26}\)

We tested this prediction further by assessing whether abolishing the SR function (using ryanodine pretreatment) could eliminate the difference in \( \text{Ca}^{2+} \) current kinetics observed in our studies. Figure 7D shows the effects of ryanodine pretreatment on the \( \text{Ca}^{2+} \)-current inactivation. \( \text{Ca}^{2+} \) currents recorded in the presence of 10 \( \mu \text{mol/L} \) ryanodine were well fit using 2 exponential functions. There were no significant differences in both \( \tau_i \) and \( \tau_t \) between the control and experimental animals after ryanodine pretreatment; \( \tau_i \) were \( 29.9±1.5 \) versus \( 28.1±2.7 \), and \( \tau_t \) were \( 125.7±3.9 \) versus \( 136.7±16.8 \); \( P=\text{NS} \) for sham \((n=9)\) as compared with banded \((n=11)\) animals at 8 weeks.

Action potentials were directly assessed before and after ryanodine in control and experimental animals (Figure 7E and 7F). Ryanodine prolonged the APD in both control and experimental animals but to a much smaller extent in the banded animals. Importantly, after abolishing the SR function, the APDs were slightly shorter in the banded animals as compared with control, possibly reflecting the increase in the \( \text{Na}^{+}-\text{Ca}^{2+} \) exchanger function as discussed below (see also Figure 8). APDs after ryanodine pretreatment at 50% and 90% repolarization (in ms) were \( 225±20 \) and \( 246±15 \) \((n=4)\) for 8-week banded and \( 261±24 \) and \( 280±24 \) \((n=3)\) for 8-week sham animals, respectively; \( P<0.05 \) comparing banded versus sham-operated animals.

Taken together, our data strongly suggest that such attenuation in the \( \text{Ca}^{2+} \)-dependent \( \text{Ca}^{2+} \) current inactivation is responsible for the observed action potential prolongation. However, other abnormalities (eg, defect in the coupling between L-type \( \text{Ca}^{2+} \) channel and the ryanodine release channel) could be invoked to explain the present findings\(^{27}\) as well.

**Figure 5. Continued.**

**Na\(^+\)-Ca\(^2+\) Exchange Current Is Upregulated in the Cardiac Hypertrophy and Failure Model**

\( \text{Na}^{+}-\text{Ca}^{2+} \) exchange current density was measured in banded animals compared with sham-operated controls. Depolarization from a holding potential of \(-80 \text{ mV} \) to positive membrane potentials elicited declining outward currents. Repolarization back to the holding potential elicited declining inward tail currents. The \( \text{Ni}^{2+} \)-sensitive currents (the \( \text{Na}^{+}-\text{Ca}^{2+} \) exchanger currents\(^{23}\)) were determined by subtracting current records obtained during exposure to 5 mmol/L \( \text{Ni}^{2+} \) from control traces. Figure 8A shows the \( \text{Ni}^{2+} \)-sensitive currents obtained from sham-operated as compared with banded animals at 4 and 8 weeks. Figure 8B shows the significant increase in the peak tail current density after depolarization to \(+60 \text{ mV} \) from animals with compensated hypertrophy and failure as compared with the age-matched sham-operated controls. The corresponding tail current density (in pA/pF) after depolarization to \(+50 \text{ mV} \) was \(-2.0±0.7 \) versus \(-8.0±1.1 \) \((n=5), P=0.01 \) comparing banded and sham animals at 4 weeks and \(-1.3±0.1 \) versus \(-0.6±0.1 \) \((n=6), P=0.02 \) comparing banded and sham animals at 8 weeks.)
Western blots were used to further establish the level of the Na\(^{+}\)-Ca\(^{2+}\) exchanger protein. Figure 8C through 8E shows a significant increase in the exchanger protein level in both hypertrophy and failure animals as compared with controls.

**Discussion**

One of the problems in the mechanistic studies of human heart failure is the inability to investigate and follow the early and longitudinal changes of the disease process. In this study, we assessed the electrophysiologic changes in a guinea pig model during an early time point of the disease, when the animals develop compensated cardiac hypertrophy, and at a later time point, when congestive heart failure has developed.

We have demonstrated the following. (1) APDs are significantly prolonged during both cardiac hypertrophy and failure as compared with the age-matched control. (2) These changes in the repolarization process are not associated with changes in the K\(^{+}\) current density. (3) There is a significant upregulation of the Na\(^{+}\) current density and Na\(^{+}\)-Ca\(^{2+}\) exchanger current density. (4) Finally, whereas there are no changes in the Ca\(^{2+}\) current density, the Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) channel is significantly attenuated in cardiac hypertrophy and failure. Changes in the inactivation kinetics of the L-type Ca\(^{2+}\) channel may result from the downregulation of the sarcoplasmic reticulum Ca\(^{2+}\) ATPase protein with a compensatory increase in the Na\(^{+}\)-Ca\(^{2+}\) exchanger. This attenuation of the inactivation kinetics of the L-type Ca\(^{2+}\) channel underlies, at least in part, the prolongation of the action potential during cardiac hypertrophy and failure.

The hemodynamic consequences of aortic banding in this model have previously been examined.\(^1\)\(^3\) No significant difference was observed in the basal nonstimulated cardiac functional parameters between the compensated hypertrophic and the respective sham-operated control animals. In contrast, contractility, developed pressure, speed of relaxation, normalized time-to-peak pressure, and normalized time to half-relaxation were depressed significantly in banded animals at 8 weeks.

**Mechanisms of Action Potential Prolongation in Cardiac Hypertrophy and Failure**

One single most consistent abnormality found in animal models of cardiac hypertrophy and failure models is APD prolongation.\(^5\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\) Prolongation of APD may occur secondary to an increase in the depolarizing currents and/or a decrease in the repolarizing currents. We and others have previously shown that downregulation of I\(_{\text{Na}}\) and I\(_{\text{K1}}\) underlie action potential prolongation in cardiac hypertrophy and failure.

**Figure 6.** L- and T-type Ca\(^{2+}\) current densities are not affected by hypertrophy or heart failure. A, Examples of L-type Ca\(^{2+}\) current traces obtained from the hypertrophy and heart failure animals compared with age-matched sham-operated animals. Test potentials used are indicated at the left of traces. Experiments were performed using 2 mmol/L external Ca\(^{2+}\). External Na\(^{+}\) was replaced by N-methyl-D-glucamine, and Ca\(^{2+}\) replaced K\(^{+}\). Currents were elicited using depolarizing voltage steps (500 ms) from −40 to +60 mV in 5-mV increments from a holding potential of −50 mV at a stimulation frequency of 0.1 Hz. Lower panels show the corresponding current density–vs-voltage relations from sham-operated (○, □) and banded (●, ■) animals at 4 and 8 weeks, respectively. Data are mean ± SEM. From each group, No. of cells = 4 to 6, and No. of animals = 3. B, Examples of T-type Ca\(^{2+}\) currents. Currents were obtained by digital subtraction of currents recorded during holding potentials of −90 vs −50 mV. Lower panels show corresponding current density–vs-voltage relations from sham-operated (○, □) and banded (●, ■) animals at 4 and 8 weeks, respectively. Data are mean ± SEM. From each group, No. of cells = 3, and No. of animals = 3.
incongruity of the results from previous studies may result in others showing a significant increase,8–10,28,29 still others showed no change in the Ca2+ current.9,33–35 These disparities are due in part to the differences in the models used.30 In addition, the incongruity of the results from previous studies may result from variation in the experimental conditions. For example, Ca2+ currents are frequently measured using high concentrations of Ca2+ chelators, which may mask the changes in the kinetics of the Ca2+ current associated with cardiac hypertrophy and failure. By using 50 μmol/L BAPTA, which is a high enough concentration to allow stable Ca2+ current recordings but substantially low to reduce the effects of exogenous Ca2+ chelators on intracellular Ca2+ handling, we observed marked differences between the inactivation kinetics of the L-type Ca2+ current in cardiac hypertrophy and failure animals as compared with age-matched control animals.

**Interdependence of Ca2+ Handling and Membrane Currents in Cardiac Hypertrophy and Failure Models**

In contrast to other models of cardiac failure, which show a significant downregulation of If and Ik, there are no significant changes in If or Ik in the guinea pig model during hypertrophy and failure stages. We propose an alternate...
mechanism of action potential prolongation in this guinea pig model of hypertrophy and failure, which is dependent on changes in Ca\(^{2+}\) cycling proteins. There is a significant downregulation of the SR Ca\(^{2+}\)-ATPase protein level (85% of the control) and phospholamban (65% of the control), coupled with a significant upregulation of the Na\(^{+}\)-Ca\(^{2+}\) exchanger protein. Such alteration in the expression of Ca\(^{2+}\) cycling proteins is predicted to decrease SR Ca\(^{2+}\) load and Ca\(^{2+}\) release, leading to a reduction in the Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) currents. Indeed, such attenuation of the Ca\(^{2+}\) current inactivation is documented in our study. A depression in the Ca\(^{2+}\) current inactivation has previously been documented in this guinea pig model during the hypertrophy stage, with further depression in the peak systolic [Ca\(^{2+}\)] in animals with evidence of congestive heart failure. This decrease in the peak systolic [Ca\(^{2+}\)], was associated with a decrease in the time to peak and relaxation of the Ca\(^{2+}\) transient.

In addition, previous studies have shown a close interaction between SR Ca\(^{2+}\) load and Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) channel. The present findings are consistent with a theoretical model, which predicts that alteration of the Ca\(^{2+}\) cycling proteins leads to a decrease in the Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) channels. Under the normal modulatory role of intracellular Ca\(^{2+}\), changes in Ca\(^{2+}\) cycling proteins have greater effects on the APD than the downregulation of \(I_{\text{Ca}}\). The reduction of \(I_{\text{Ca}}\) leads to a significant prolongation of the APD only under conditions of exogenous intracellular Ca\(^{2+}\) buffering, which masks the modulatory effects of Ca\(^{2+}\) and enhances the influence of the outward K\(^{+}\) current. By recording action potential using a perforated patch, we expect the modulatory role of the endogenous intracellular Ca\(^{2+}\) to be operable.

We have found a compensatory upregulation of the Na\(^{+}\)-Ca\(^{2+}\) exchange current and the protein level. Similar changes in the exchanger levels have previously been shown in a canine tachycardia-induced heart failure and human heart failure. This alternate Ca\(^{2+}\) removal system by Na\(^{+}\)-Ca\(^{2+}\) exchanger may compensate for the defective SR Ca\(^{2+}\) uptake. In addition, the enhanced Ca\(^{2+}\) entry via the reverse mode of the Na\(^{-}\)-Ca\(^{2+}\) exchanger may provide inotropic support for the failing myocytes. Because the exchanger is electrogenic, it also plays a significant role in the action potential profile. The increase in the exchanger current alone may shorten the APD; however, these changes may be counteracted by the increase in the inward Ca\(^{2+}\) currents.

### Upregulation of the Na\(^{+}\) Current Density

Na\(^{+}\) current density was significantly increased during cardiac hypertrophy and failure, but there were no changes in the steady-state activation and inactivation kinetics. The exact
mechanisms of this upregulation are not known. However, such changes may arise from an increase in the channel biosynthesis resulting from changes in the intracellular Ca\textsuperscript{2+}, as previously reported.\textsuperscript{46–48} The amplitude of the Ca\textsuperscript{2+} transient is depressed in several models of cardiac hypertrophy and failure, including this guinea pig model.\textsuperscript{26,43,49–51} Changes in Na\textsuperscript{+} current density have been documented in different in vivo animal models. There was a significant reduction in the Na\textsuperscript{+} current density in cardiac myocytes isolated from the epicardial border zone surrounding the infarcted area as compared with the normal zone in infarcted canine hearts, which may result from intracellular Ca\textsuperscript{2+} overload in the cardiac myocytes in these cells.\textsuperscript{52} Similarly, alteration of Na\textsuperscript{+} channel expression has been demonstrated in a dog model of atrial fibrillation.\textsuperscript{53} These data suggest that Na\textsuperscript{+} channel expression may be tightly regulated by changes in intracellular Ca\textsuperscript{2+}.

Finally, the present study indicates that changes that occur during cardiac hypertrophy parallel those that occur during the later stage when heart failure has developed. Evaluation at an even earlier time point may be necessary to follow the progression from cardiac hypertrophy to failure. The present study suggests a continuum of changes in the action potential characteristic and the level of the Ca\textsuperscript{2+} cycling proteins in this model of cardiac hypertrophy and failure. There is a strong interdependence between the abnormality in Ca\textsuperscript{2+} cycling proteins found in cardiac hypertrophy and failure and the electrophysiological changes in these states.

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References

3. Cohn J, Archibald D, Ziesche S, Franciosa J, Harston W, Tristani F, Ahmmed et al Cardiac Hypertrophy and Failure 569

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