Enhanced Ca\(^{2+}\) Release and Na/Ca Exchange Activity in Hypertrophied Canine Ventricular Myocytes

Potential Link Between Contractile Adaptation and Arrhythmogenesis

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Background—Ventricular arrhythmias are a major cause of sudden death in patients with heart failure and hypertrophy. The dog with chronic complete atrioventricular block (CAVB) has biventricular hypertrophy and ventricular arrhythmias and is a useful model to study underlying cellular mechanisms. We investigated whether changes in Ca\(^{2+}\) homeostasis are part of the contractile adaptation to CAVB and might contribute to arrhythmogenesis.

Methods and Results—in enzymatically isolated myocytes, cell shortening, Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR), and SR Ca\(^{2+}\) content were enhanced at low stimulation frequencies. Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels was unchanged, but Ca\(^{2+}\) influx via the Na/Ca exchanger was increased and contributed to Ca\(^{2+}\) loading of the SR. Inward Na/Ca exchange currents were also larger. Changes in Ca\(^{2+}\) fluxes were less pronounced in the right versus left ventricle.

Conclusions—Enhanced Na/Ca exchange activity may improve contractile adaptation to CAVB but at the same time facilitate arrhythmias by (1) increasing the propensity to Ca\(^{2+}\) overload, (2) providing more inward current leading to (nonhomogeneous) action potential prolongation, and (3) enhancing (arrhythmogenic) currents during spontaneous Ca\(^{2+}\) release. (Circulation. 2000;102:2137-2144.)

Key Words: calcium ■ myocytes ■ sarcoplasmic reticulum ■ hypertrophy

Enhanced susceptibility to arrhythmias in hypertrophy and heart failure is due to structural and functional, including electrical, remodeling. Disturbances in repolarization, with QT prolongation and enhanced dispersion of repolarization, provide the substrate for ventricular arrhythmias.\(^1\,2\) Understanding the underlying cellular and molecular changes is a major goal.\(^3\) For end-stage heart failure, important insights have come from studies of tissues and myocytes from human hearts as well as from animal models. Regionally variable reductions in the transient outward K\(^+\) current, \(I_{\text{to}}\,3-5\) as well as other membrane currents (reviewed, eg, in Reference 6), contribute to changes in the action potential profile. Changes in [Ca\(^{2+}\)], homeostasis may be important as well.\(^7\,8\)

Human data on remodeling during compensated hypertrophy are scarce because obtaining tissues is a major obstacle. Several animal models of hypertrophy (see, eg, References 2 and 6) made it possible to study [Ca\(^{2+}\)], homeostasis and ionic currents but also had a number of limitations. Very often, a depressed function or decrease in systolic [Ca\(^{2+}\)], transients was found (eg, References 9 through 11, but see also Reference 12). Probably, some of these animal models may have early progression to heart failure, complicating the study of compensated hypertrophy (see also Reference 13). Another shortcoming is the difficulty of relating cellular changes to in vivo arrhythmic events. Larger animals are more relevant for human arrhythmogenesis but are less available for cellular studies.

The dog with chronic complete atrioventricular block (CAVB) develops biventricular hypertrophy, but cardiac function in vivo at 6 to 9 weeks of AVB is not impaired. At this stage, the dog is more susceptible to triggered arrhythmias, related to prolongation and increased dispersion of repolarization, and the occurrence of afterdepolarizations.\(^14\) A decrease in delayed outward rectifier K\(^+\) current was implicated in the action potential prolongation of the hypertrophied myocytes,\(^15\,16\) indicating ionic remodeling. Therefore, this model offers a unique opportunity to further investigate the cellular mechanisms of arrhythmias in compensated hypertrophy.

In an accompanying study,\(^16\) establishment of the link between the occurrence of delayed afterdepolarization–dependent arrhythmias in vivo and enhanced contractile function with CAVB. In the present study, we investigate the hypothesis that cellular changes in Ca\(^{2+}\) transport mechanisms are responsible for the preserved contractile function but may also be implicated in the arrhythmogenesis.
Methods

Cell Isolation
A total of 18 dogs were studied after 8±1 weeks of CAVB. A group of 15 dogs in sinus rhythm, with comparable body weights, served as controls. The procedure for cell isolation has been described in detail before.\(^\text{15,17}\) For all CAVB dogs, hypertrophy was confirmed by a heart weight/body weight ratio of 11.4±0.4 versus 8.0±0.2 for control dogs (\(P<0.001\)).

Recording of Cell Shortening, Membrane Currents, and [Ca\(^{2+}\)]
All experiments were performed at 36°C to 37°C. Cells were studied on an inverted microscope (Nikon Diaphot). Shortening of cells impaled with high-resistance microelectrodes (30 to 60 M\(\Omega\)) was measured with a video edge detector (Crescent Electronics).

Membrane currents were recorded during whole-cell ruptured patch clamp with an Axopatch 1D amplifier, filtered at 1 kHz, and sampled and digitized at 4 kHz (Fastlb45, Indec Systems). Cell capacitance was 155±6 pF (\(n=48\)) in CAVB left ventricular (LV) myocytes versus 135±9 pF (\(n=39\)) in controls (\(P<0.05\)), and the capacitance of right ventricular (RV) myocytes was 150±11 pF (\(n=30\)) in CAVB versus 109±6 pF (\(n=39\)) in controls (\(P<0.05\)). [Ca\(^{2+}\)] was monitored with fluo 3 (50 µmol/L) or a combination of fluo 3 (30 µmol/L) and fura red (70 µmol/L). The setup and calibration were as described before.\(^\text{18}\) Resting [Ca\(^{2+}\)] was determined from data obtained with the dual-dye method. With fluo 3 alone, fluorescence values were background-corrected and normalized from data obtained with the dual-dye method. With fluo 3 and fura red, fluorescence values were background-corrected and normalized for baseline fluorescence (pseudoratio); [Ca\(^{2+}\)] was calculated assuming a \(K_d\) of 400 mmol/L and a resting value of 100 mmol/L.\(^\text{19}\) Values obtained with this latter approach correlated well with the calibrated values obtained with the dual-dye method.

Pooled data are shown as mean±SEM. Comparisons between groups were made with Student’s unpaired \(t\) test or a \(\chi^2\) test where appropriate. Values of \(P<0.05\) were considered significant.

Solutions and Experimental Protocols
The pipette solution contained (in mmol/L): K-aspartate 120, KCl 20, K-HEPES 10, MgATP 5, MgCl\(_2\) 0.5, NaCl 10 or 20, fluo 3 0.05 (or fluo 3 0.03 and fura red 0.07); pH 7.20. The external solution contained (in mmol/L): NaCl 130, KCl 5.4, Na-HEPES 11.8, MgCl\(_2\) 0.5, CaCl\(_2\) 1.8, glucose 6; pH 7.35. In some experiments, K-aspartate and KCl were replaced with Cs-aspartate and CsCl, respectively.

To determine sarcoplasmic reticulum (SR) Ca\(^{2+}\) content, we measured the amount of Ca\(^{2+}\) extruded by the Na/Ca exchanger during caffeine-induced discharge of the SR,\(^\text{20}\) after a series of 300-ms depolarizing pulses (from −70 to +10 mV) at either 0.25 Hz or 1 Hz. Data are expressed as mmol/L accessible cell volume,\(^\text{21}\) assuming a surface/volume ratio of 5 pF/µL for dog myocytes and 0.65 accessible volume.

To measure L-type Ca\(^{2+}\) current (\(I_{\text{CaL}}\)), K\(^+\) and Na\(^+\) in all solutions was replaced with tetraethylammonium (TEA\(^-\)) and N-methyl-D-glucamine (NMDG\(^+\)), and [Ca\(^{2+}\)] was buffered with 10 mmol/L EGTA.

To block \(I_{\text{CaL}}\), we used 20 µmol/L nisoldipine (Bayer) or 20 µmol/L nifedipine (Sigma). Ca\(^{2+}\) release from the SR was disabled with 10 µmol/L ryanodine (Sigma). In the presence of nisoldipine and ryanodine, NiCl\(_2\) (2.5 mmol/L) was used to block Na/Ca exchange. Ni\(^{2+}\)-sensitive currents were measured at 30 ms of depolarization to minimize interference of time-dependent changes or K\(^+\) currents. Current densities measured with all K\(^+\) replaced with Cs\(^+\) were not different from those with K\(^+\) (1.25±0.62 versus 1.23±0.65 pA/pF at +70 mV in each group of 3 cells from the same heart), as previously reported.\(^\text{18}\)

Results

Contractile Function In Vitro
In single LV cells from control dogs, increasing the frequency of stimulation tended to increase the extent of shortening (\(P=\text{NS}\), Figure 1). In cells from CAVB dogs, the extent of shortening was significantly larger than in controls at frequencies <120 bpm (\(P<0.05\)) and tended to decrease with increasing frequencies.

[Ca\(^{2+}\)] Transients and Ca\(^{2+}\) Content of the SR
Action potentials and [Ca\(^{2+}\)], transients during whole-cell recording are illustrated in Figure 2. Resting [Ca\(^{2+}\)], was not different for cells from control and from CAVB dogs (108±20 versus 110±7 mmol/L, respectively). In cells from control dogs, the frequency dependence of [Ca\(^{2+}\)], transients, as for cell shortening, was shallow. In CAVB cells, the [Ca\(^{2+}\)] transients were largest at low frequencies and significantly larger than in control cells (Figure 3A). Significant differences in [Ca\(^{2+}\)], were already present during the rapid upstroke (Figure 3B), indicating that early Ca\(^{2+}\) release from the SR was larger. This was not dependent on a more pronounced increase in action potential duration at the lower frequencies in CAVB,\(^\text{15}\) because it was maintained during stimulation at different frequencies in voltage-clamp mode with a fixed pulse duration (results not shown).

We next examined whether the increased Ca\(^{2+}\) release of CAVB cells could be related to an increase in Ca\(^{2+}\) content of the SR (Figure 3C). The frequency dependence of SR Ca\(^{2+}\) content of control and of CAVB cells reflected the frequency dependence of the [Ca\(^{2+}\)], transients. In CAVB cells, Ca\(^{2+}\) content at 0.25 Hz was significantly larger than in control cells; at 1 Hz, the content also tended to be larger, but the difference was not statistically significant (\(P=0.059\)), possibly because of scatter.
We then examined the possible sources of increased Ca$^{2+}$ influx or decreased Ca$^{2+}$ efflux that could explain the increased Ca$^{2+}$ content of the SR.

Properties of the L-type Ca$^{2+}$ Current, $I_{CaL}$

$I_{CaL}$ was studied in a separate series of experiments (Figure 4). The amplitude of the peak currents was similar for LV cells from control and from CAVB dogs, and the I-V relations were not shifted. Steady-state inactivation properties were unchanged.

Ca$^{2+}$ Influx via the Na/Ca Exchanger

Ca$^{2+}$ influx via the exchanger in LV cells was measured in the absence of $I_{CaL}$ and of Ca$^{2+}$ release from the SR; pipette [Na$^+$] was 20 mmol/L (Figure 5A). During depolarization, [Ca$^{2+}$]$_i$ rose slowly. On repolarization to $-45$ mV, a [Ca$^{2+}$]-$dependent$ inward tail current (not sensitive to Cl channel blockers) was recorded, related to Ca$^{2+}$ efflux through the exchanger. The increase in [Ca$^{2+}$]$_i$ was significantly larger in cells from CAVB than in control cells (Figure 5B), as was the inward tail current (Figure 5C).

Amplitude of Na/Ca Exchange Currents

Ni$^{2+}$-sensitive outward Na/Ca exchange currents were measured in the same experimental conditions (Figure 6). Consistent with the larger increase in [Ca$^{2+}$]$_i$, outward current densities were significantly larger for CAVB. In the negative potential range, effects of Ni$^{2+}$ on $I_{K1}$ may pose a problem, and reversal potentials were hard to determine. With Cs$^+$ substituted for K$^+$, a clear inward exchanger current was seen at the prepulse potential of $-50$ mV in control cells ($-0.51 \pm 0.25$ pA/pF, $n_{cells}=5$) but not in cells from CAVB ($0.02 \pm 0.15$ pA/pF, $n_{cells}=5$), suggesting that CAVB cells had a more negative reversal potential at similar resting values of [Ca$^{2+}$].

The current density of the inward tail current on repolarization as a function of [Ca$^{2+}$], was larger for CAVB (Figure 6C).

Reverse Mode Na/Ca Exchange as a Trigger for SR Ca$^{2+}$ Release

We also examined whether Na/Ca exchange could act as a trigger for SR Ca$^{2+}$ release in CAVB (with block of $I_{CaL}$ and pipette [Na$^+$] 20 mmol/L). In these conditions, the upstroke of [Ca$^{2+}$]$_i$ was significantly delayed and slower (Figure 7A). This Na/Ca exchange–dependent release (ryanodine-sensitive, not shown) was observed more frequently in CAVB cells, and the amplitude of [Ca$^{2+}$]$_i$ transients on average was larger (Figure 7B and 7C).

Possible Contribution of Changes in [Ca$^{2+}$] and Na/Ca Exchange to Arrhythmogenesis

To investigate potential regional heterogeneity, studies as described above were also performed in RV cells. As for LV...
cells, the amplitude of $[Ca^{2+}]_i$ transients was larger at low frequencies of stimulation: at 0.25 Hz, $[Ca^{2+}]_i$ was $371 \pm 64$ nmol/L for CAVB, $n_{cells}=10$, $n_{dogs}=4$ versus $277 \pm 26$ nmol/L for control, $n_{cells}=8$, $n_{dogs}=3$ ($P<0.05$), but the increase was smaller than for the LV cells ($507 \pm 97$ nmol/L in CAVB versus $266 \pm 23$ nmol/L in control). $I_{CaL}$ density of RV cells was unchanged with CAVB and comparable to values for LV cells. $Ca^{2+}$ influx via the Na/Ca exchange was significantly increased for RV CAVB cells compared with controls; eg, for pulses to $+40$ mV, $[Ca^{2+}]_i$, at the end of the pulse was $365 \pm 77$ nmol/L for CAVB ($n_{cells}=11$, $n_{dogs}=5$) versus $234 \pm 46$ nmol/L for controls ($n_{cells}=12$, $n_{dogs}=6$, $P<0.05$). This increase was slightly less than in LV cells ($[Ca^{2+}]_i$, at $+40$ mV in LV CAVB cells was $423 \pm 76$ nmol/L). Ni$^{2+}$-sensitive outward currents were significantly larger in RV CAVB cells compared with controls; eg, for pulses to $+40$ mV, $[Ca^{2+}]_i$, at the end of the pulse was $365 \pm 77$ nmol/L for CAVB ($n_{cells}=11$, $n_{dogs}=5$) versus $234 \pm 46$ nmol/L for controls ($n_{cells}=12$, $n_{dogs}=6$, $P<0.05$). This increase was slightly less than in LV cells ($[Ca^{2+}]_i$, at $+40$ mV in LV CAVB cells was $423 \pm 76$ nmol/L).

We also tested whether the larger $Ca^{2+}$ influx via the exchanger of CAVB cells could induce $Ca^{2+}$ overload and (potentially arrhythmogenic) spontaneous SR $Ca^{2+}$ release. With 10 mmol/L NaCl in the pipette and with $I_{CaL}$ blocked, $Ca^{2+}$ release and $[Ca^{2+}]_i$ oscillations indicative for SR $Ca^{2+}$ overload could be induced during long pulses to positive potentials (Figure 8B). Spontaneous release was observed more frequently in CAVB cells than in control cells (for steps to $+50$ mV in 10 of 12 CAVB cells versus 1 of 8 control cells, $P<0.01$).

**Discussion**

**Contractile Function and Enhanced SR Ca$^{2+}$ Release in the Dog With CAVB**

Measurements of LV $dP/dt$ in vivo indicated that contractile function after 6 weeks of AVB is better than in the acute stage of AVB, in particular at low stimulation rates.$^{16b}$ Our measurements of cell shortening confirm this finding. The “inversion” of the $[Ca^{2+}]_i$-frequency relation observed in the present study should be contrasted to the findings in human heart failure, in which the inversion results from a loss of contraction at the higher rates, with (near) normal function at the low frequencies.$^{22}$ The enhanced contractile function at
the slow idioventricular rate maintains cardiac output in CAVB. Our present findings are therefore of particular importance because they report on a stage of compensated hypertrophy in which an increased susceptibility to arrhythmias has been reported in patients.1

Mechanisms of Increased SR Ca2+ Release in CAVB

In CAVB, SR Ca2+ release and SR Ca2+ content are increased at the lower stimulation frequencies. Ca2+ influx through the L-type Ca2+ channel was found to be unchanged. In contrast, Ca2+ influx through the exchanger was significantly enhanced in CAVB. However, Ca2+ efflux was also larger in CAVB. To explain the increased Ca2+ release at low frequencies, we therefore have to assume that at these frequencies, the increased influx is dominant, whereas at higher frequencies, influx and efflux are more balanced. The precise mechanisms of this preferential loading at low frequencies remains to be elucidated, but the latter is reminiscent of the behavior of cardiac muscle with increased intracellular [Na+] (see, eg, Reference 23). Although our data indicate that Ical is the predominant trigger for Ca2+ release, the increased Ca2+ influx through the Na/Ca exchanger may facilitate this trigger mechanism.24 Thus, increased Ca2+ influx via the exchanger in CAVB leads to increased Ca2+ release through an increase in SR Ca2+ content, with perhaps a small contribution of an increase in trigger Ca2+.

Increased Activity of the Na/Ca Exchanger: Upregulation and/or Changes in Reversal Potential

When measured directly, Ca2+ influx via the exchanger and outward Na/Ca exchange currents were more than 2-fold larger in CAVB. Such an increase could result from higher expression levels, expected to produce a nearly equal increase...
in inward currents. Although the inward Na/Ca exchange current for LV cells was larger, the increase was less than for the outward current. One possible explanation is a concomitant negative shift of the reversal potential in CAVB, as supported by the presence of a net negative inward current at -50 mV in control but not in CAVB cells. Such a shift could be related to an increase in subsarcolemmal [Na\(^+\)]. This hypothesis is attractive, because higher cytoplasmic [Na\(^+\)] has been associated with a preferential increase in Ca\(^{2+}\) release at low frequencies of stimulation, and because there is a well-established link between an increase in [Na\(^+\)] and the occurrence of spontaneous Ca\(^{2+}\) release.

Can the Adaptive Process Contribute to Arrhythmogenesis?

In the dog with CAVB, as in human ventricular remodeling, increased regional dispersion and prolongation of repolarization are the substrate for ventricular arrhythmias, triggered by ectopic beats, related to afterdepolarizations or local reentry. The increase in Na/Ca exchange described in the present study is likely to be an important factor in the arrhythmia substrate. Inward Na/Ca exchange current contributes to the plateau of the action potential duration, and enhanced Ca\(^{2+}\) release in CAVB together with increased current density will lead to larger inward Na/Ca exchange currents, prolonging the action potential. This will be more pronounced in the LV, because [Ca\(^{2+}\)], and the inward exchanger current are larger, thus contributing to regional dispersion. Our present findings may also shed new light on cellular events responsible for the initiation of arrhythmias. Torsades de pointes can be evoked in the dog with CAVB in the presence of class III antiarrhythmics. Early afterdepolarizations precede their onset.

Although in vitro these early afterdepolarizations are not associated with spontaneous Ca\(^{2+}\) release, the Na/Ca exchanger may provide the necessary inward current to facilitate depolarizing window Ca\(^{2+}\) currents. A more direct role for increased Ca\(^{2+}\) release and exchanger current can be inferred for arrhythmias evoked by pacing in the absence of class III agents. The occurrence of delayed afterdepolarizations and ectopic beats appears to be related to a critical increase in LV dP/dt, which can be obtained in CAVB but not in the acute stage of AVB. Because the amplitude of the Na/Ca exchange current will be directly related to the contraction and underlying [Ca\(^{2+}\)], transient, this current may provide the dynamic component for the initiation of the ectopic beats during spontaneous Ca\(^{2+}\) release.

**Perspectives**

Our data support the newly emerging idea that not only forward- but also reverse-mode Na/Ca exchange may be important in ventricular remodeling. Increased Ca\(^{2+}\) influx via the exchanger, contributing to Ca\(^{2+}\) loading of the SR, was recently described in the rabbit heart after myocardial infarction. In human heart failure, the Na/Ca exchanger also appears to be very important in determining the SR Ca\(^{2+}\) load, a notion supported by the finding that increasing Na\(^+\) influx enhances contractile function to a larger extent in myocardium from heart failure patients than from controls.

Increased exchanger activity has been reported for some animal models of hypertrophy and heart failure, but not all, suggesting that eventual changes, as in the dog with CAVB, represent a specific response associated with a particular type of Ca\(^{2+}\) handling. Consistent with this idea, expression levels of Na/Ca exchange have recently been...
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mechanisms of sudden death in patients with compensated
ventricular hypertrophy.

Conclusions
During compensated hypertrophy in the dog with CAVB, enhanced Na/Ca exchange activity may preserve contrac-
tile function by increasing Ca\textsuperscript{2+} release at the lower heart
rates. This adaptive mechanism, however, may at the same
time contribute to arrhythmogenesis by increasing the
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