Ionic Remodeling of Cardiac Purkinje Cells by Congestive Heart Failure

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Background—Cardiac Purkinje cells (PCs) are important for the generation of triggered arrhythmias, particularly in association with abnormal repolarization. The effects of congestive heart failure (CHF) on the ionic properties of PCs are unknown.

Methods and Results—PCs were isolated from false tendons of control dogs and dogs with ventricular tachypacing-induced CHF. CHF PCs were hypertrophied (capacitance, mean±SEM, 149±4 pF, n=130; versus 128±3 pF, n=150, control; P<0.001). Transient outward current density was reduced in CHF PCs without change in voltage dependence or kinetics. CHF also reduced inward-rectifier current density, with no change in form of the current-voltage relationship. Densities of L- and T-type calcium, rapid and slow delayed rectifier, and Na⁺-Ca²⁺ exchange currents were unaltered by CHF, but L-type calcium current inactivation was slowed at positive potentials. Purkinje fiber action potentials from CHF dogs showed decreased phase 1 amplitudes and elevated plateau voltages and demonstrated twice as much prolongation on exposure to the rapid delayed rectifier blocker E-4031 as control Purkinje fibers.

Conclusions—CHF causes remodeling of important K⁺ and Ca²⁺ currents in cardiac PCs, decreasing repolarization reserve and causing an exaggerated repolarization delay in response to a class III drug. These results have important potential implications regarding ventricular arrhythmogenesis, particularly related to triggered activity in PCs, in patients with CHF. (Circulation. 2001;104:2095-2100.)

Key Words: ion channels • remodeling • electrophysiology • antiarrhythmia agents • heart failure

Congestive heart failure (CHF) predisposes to the generation of ventricular tachyarrhythmias1 and the occurrence of sudden death.2 In addition, CHF promotes drug-induced Torsades de Pointes arrhythmias.3 Abnormal repolarization, related to ion channel remodeling, is important in the arrhythogenic potential of CHF.1,4,5 CHF-induced remodeling of ionic currents in ventricular6 and atrial7 myocytes has been studied in detail. Cardiac Purkinje cells (PCs) are believed to play important roles in the generation of ventricular arrhythmias, particularly those related to triggered activity.7–9 Ionic currents are altered in subendocardial PCs in regions of myocardial infarction10–12; however, almost nothing is known about PC ionic remodeling in CHF. The present study was designed to evaluate CHF-induced changes in ionic currents and action potentials (APs) in canine cardiac PCs.

Methods

CHF Preparation

CHF was produced as previously described6 by pacing the right ventricle at 240 bpm for 3 weeks followed by 2 weeks at 220 bpm. Dogs were then anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV load, 29.25 mg/kg per hour infusion), and a median sternotomy was performed. All animal care and handling procedures followed the guidelines of the Canadian Council on Animal Care. Excised hearts were immersed in Tyrode solution at room temperature. Free-running false tendons were excised into modified Eagle’s MEM (Gibco-BRL; pH 6.8, HEPES-NaOH) containing collagenase (800 to 900 U/mL, Worthington Type-II) and 1% BSA (Sigma), and single PCs were isolated as previously described13,14.

Solutions

Standard Tyrode solution contained (in mmol/L) NaCl 136, KCl 5.4, MgCl₂ 1, CaCl₂ 1, NaH₂PO₄ 0.33, HEPES 5, and dextrose 10; pH 7.4 (NaOH). High-K⁺ storage solution contained (in mmol/L) KCl 20, KH₂PO₄ 10, dextrose 10, glutamic acid 70, β-hydroxybutyric acid 10, taurine 10, and EGTA 10; 0.1% BSA; pH 7.4 (KOH). Standard pipette solution contained (in mmol/L) K⁺ aspartate 110, KCl 20, MgCl₂ 1, Mg₂ATP 5, HEPES 10, phosphocreatine 5, GTP 0.1, and EGTA 5; pH 7.2 (KOH). Solutions were equilibrated with 100% O₂.

For K⁺ current measurement, the extracellular solution included 1 μmol/L atropine to eliminate muscarinic K⁺ currents and CdCl₂ (200 μmol/L) or nimodipine (1 μmol/L, for Iₖs studies) to block Ca²⁺ currents. Na⁺ current contamination was prevented by equimolar substitution of choline for extracellular Na⁺. For currents other than transient outward current (Iₒ), 1 mmol/L 4-AP was used to block Iₒ. Rapid delayed rectifier current (Iₖ) was studied as 5 μmol/L E4031-sensitive current and inward rectifier current (Iₖᵥ) as 1 mmol/L Ba²⁺-sensitive current. Slow delayed rectifier current (Iₖ)
was studied in the presence of 1 μmol/L doxetilide to eliminate \( I_{K1} \). For \( I_{K1} \) recording, the bath solution contained tetraethylammonium chloride, CsCl, and CsOH in place of NaCl, KCl, and NaOH, respectively, and [CaCl\(_2\)] was 2 mmol/L. The pipette for \( I_{K1} \) recording contained (in mmol/L) CsCl 20, Cs-aspartate 110, MgCl\(_2\) 1, EGTA 5, pH 7.2 (CsOH). Na\(^+\)-Ca\(^{2+}\) exchange (NCX) current (\( I_{\text{NCX}} \)) was recorded with ramp pulses and extracellular (in mmol/L, NaCl 140, CaCl\(_2\) 0 or 5, MgCl\(_2\) 1, CsCl 5, HEPES 5, n-midipine 0.001, ouabain 0.01, and ryanodine 0.005; pH 7.2 CsOH) and pipette (in mmol/L, CsCl 90, NaCl 50, MgATP 5, MgCl\(_2\) 3, EGTA 20, CaCl\(_2\) 13, and HEPES 20; pH 7.2, CsOH) solutions designed to suppress \( K^+ \) current, Na\(^+\)-K\(^+\) ATPase, and sarcoplasmic reticulum Ca\(^{2+}\) release. 15

Data Acquisition and Analysis
Whole-cell patch clamp was performed as previously described\(^{13,14} \) at 36.5°C. Compensated series resistance and capacitive time constants (\( t_s \)) averaged 2.5±0.1 M\( \Omega \) and 290±10 μs. Leakage compensation was not used. The capacitance of CHF cells was increased (149±4 pF, \( n=130 \), versus 128±6 pF in control, \( n=150; P<0.001 \)), so currents are expressed in terms of density.

Standard microelectrode techniques were used to record action potentials (APs). The Tyrode solution contained (in mmol/L) NaCl 120, KCl 1.5, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 0.1, NaHCO\(_3\) 25, CaCl\(_2\) 1.25, and dextrose 5; pH 7.4. Purkinje fiber false tendons were superfused with oxygenated (95% O\(_2\), and 5% CO\(_2\)) Tyrode solution at 36°C and impaled with 3 mol/L KCl-filled glass microelectrodes (8 to 20 M\( \Omega \)) connected to a high input–impedance amplifier.

Nonlinear least-square curve-fitting algorithms were used for curve fitting. Nonpaired \( t \) tests were used to compare CHF with control cells. \( P<0.05 \) was considered to indicate statistical significance. Group data are expressed as mean±SEM.

Results

Changes in Ionic Currents

\( K^+ \) Currents

Figure 1 shows representative \( I_{K1} \) recordings (panels A and B). CHF significantly reduced \( I_{K1} \) (C), including the outward component (D), without altering the form of the \( I_{K1} \) current-voltage relation (Figure 1C, inset).

Figures 2A and 2B illustrate \( I_{K1} \) recordings from control and CHF PCs. \( I_{K1} \) density was significantly smaller in CHF PCs (Figure 2C); however, there were no differences in the form of the \( I_{K1} \)-V relation (Figure 2D). The voltage dependence of \( I_{K1} \) inactivation was tested with a 2-pulse protocol as described in Figure 2E. Activation voltage dependence was evaluated from the relation \( I_{\text{TP}}=a_TG_{\text{max}}(V_{\text{TP}}-V_R) \), where \( I_{\text{TP}} \) and \( a_T \) are current and activation variable at test potential \( V_{\text{TP}} \). \( V_R \) is reversal potential, and \( G_{\text{max}} \) is maximal conductance. Half-maximal voltage (\( V_{1/2} \)) and activation slope factor (Boltzmann fits) were 8.9±0.7 and 10.3±0.4 mV (control) and 8.9±1.0 and 10.9±1.0 mV (CHF, \( P=NS \)). \( V_R \) obtained from the reversal of \( I_{K1} \) tail currents after 2-ms activating pulses averaged -75.3±2.2 mV. Inactivation \( V_{1/2} \) and slope factor were -30±2 and 11±1 mV (control) and -31±2 and 11±1 mV (CHF, \( P=NS \)). \( I_{K1} \) inactivation kinetics were biexponential, with \( rs \) unaltered by CHF (Figure 2F). \( I_{K1} \) recovery
Figure 3. A, Representative E-4031–sensitive \( I_{Kr} \) recordings. B, Mean ± SEM \( I_{Kr} \) density voltage relations (n = 11; control; n = 9, CHF). C, Mean ± SEM normalized \( I_{Kr} \) tail currents (n = 5 cells/group) and best-fit Boltzmann relations. D, Representative \( I_{Kr} \) recordings with 4-second depolarizing pulses (0.1 Hz) and 2-second repolarizations to −40 mV to record tail currents. E, Mean ± SEM \( I_{Kr} \) density voltage relations (n = 30; control; n = 25, CHF). F, Normalized \( I_{Kr} \) tail \( V_{TP} \) relations and best-fit Boltzmann relations. CTL indicates control; TP, test potential.

(Figure 2G) was biexponential, with \( \tau \) averaging 42±8 and 1391±67 ms (control) and 52±11 and 1486±108 ms (CHF, n = 10 for each, \( P = \text{NS} \)). \( I_{Kr} \) frequency dependence was similarly unaffected by CHF (Figure 2H).

Figure 3 shows results for \( I_{Kr} \) (left) and \( I_{Kr} \) (right). Representative control recordings are shown at the top, with mean step \( I-V \) relations, which were unchanged by CHF, in the middle. Activation voltage dependence based on normalized tail currents is shown in bottom panels and was not altered by CHF for either \( I_{Kr} \) or \( I_{Kr} \).

\( \text{Ca}^{2+} \) Currents

Representative L-type calcium current (\( I_{Ca,L} \)) recordings are shown in Figures 4A and 4B and point to slowed \( I_{Ca,L} \) decay in CHF. \( I_{Ca,L} \) density was not significantly different between control and CHF cells (Figure 4C). Inactivation \( \tau \) were slowed significantly by CHF at voltages positive to +10 mV (Figure 4D). For example, at +20 mV, \( \tau_{inact} \) increased from 6.0±0.3 to 8.2±0.9 ms (\( P < 0.05 \)) and \( \tau_{slow} \) from 38±2 to 66±8 ms (\( P < 0.01 \)) in CHF PCs. In addition to a slowing of inactivation \( \tau \), CHF significantly increased the proportion of slow-phase inactivation at positive voltages (Figure 4E). For example, at +30 mV, \( \tau \) accounted for 36±4% of inactivation in control PCs compared with 51±5% in CHF (\( P < 0.05 \)). \( I_{Ca,L} \) inactivation voltage dependence was assessed with a 2-pulse protocol (Figure 4F). Inactivation \( \tau_{slow} \) and slope factor were −25±2 and −6±1 mV (control) and −26±1 and −7±1 mV (CHF, n = 10 cells/group, \( P = \text{NS} \)). Activation voltage dependence was assessed according to the relation \( I_{V_T}=aV_TG_{max} \) (\( V_{T}=V_T \)), with \( V_T \) obtained from a linear fit to the ascending portion of the \( I-V \) relation. Resulting mean \( V_{1/2} \) and slope factors were 2.0±1.4 and 6.6±2.0 mV (control) and 0.4±1.4 and 6.5±0.1 mV (CHF, n = 10/group, \( P = \text{NS} \)). Reactivation kinetics at holding potential (HP) (−80 mV) close to the PC resting potential are shown in Figure 4G. Reactivation was monoequilibrium, with similar \( \tau \) in control (44±7 ms) and CHF PCs (53±6 ms, n = 10/group, \( P = \text{NS} \)). \( I_{Ca,L} \) showed little frequency dependence between 1 and 5 Hz at a HP of −80 mV, and there were no significant CHF-related differences in \( I_{Ca,L} \) frequency dependence (Figure 4H).
Ca.T reactivation

An example, at

and there were no significant differences between groups; for

during 250-ms depolarizations (0.1 Hz) from a HP of

mean SEM (n 10/group); curves are best-fit monoexponen-

al trials. Data are mean SEM (24 cells/group) ITP indicates control; RP, ramp potential.

NCX

Figure 6A shows INCX as determined from current during ramp depolarizations from −60 to +50 mV in the presence of 5 and 0 mmol/L [Ca\(^{2+}\)]. Reverse-mode INCX is substantial in the presence of 5 mmol/L Ca\(^{2+}\) and is absent in the presence of 0 mmol/L Ca\(^{2+}\), allowing INCX to be calculated from the difference between the two current recordings.\(^{15}\) The results in Figure 6B show that INCX amplitude was larger in CHF cells but that after correction for cell size (capacitance normalization), there were no significant differences.

Changes in APs

PC AP characteristics were first recorded at a total of 42 sites from 11 control dogs and 37 sites in 8 CHF dogs at 1 Hz. Phase 1 repolarization was less marked in CHF, and the

All PCs possessed a relatively large T-type calcium current (ICa.T) (Figures 5A and B). ICa.T was obtained as previously described\(^{16,17}\) by digital subtraction of ICa elicited at HP −50 mV from ICa at a HP of −90 mV. The mean ICa.T density voltage relation was not altered by CHF (Figure 5C). ICa.T inactivation V1/2 and slope factors averaged −61 ± 2 and 4.1 ± 0.4 mV (control) and −63 ± 2 and 4.9 ± 0.5 mV (CHF, n = 10/group, P = NS) (Figure 5D). Activation V1/2 and slope factors were −32 ± 1 and 10 ± 1 mV (control) and −30 ± 2 and 12 ± 1 mV (CHF), respectively (n = 10/group, P = NS). The time course of ICa.T inactivation was monoexponential, and there were no significant differences between groups; for example, at −20 mV (voltage of maximum ICa.T), τs were 4.5 ± 0.2 ms (control, n = 10) versus 4.6 ± 0.3 ms (CHF, n = 10, P = NS). ICa.T reactivation was assessed (Figure 5E) at a test potential (−30 mV) at which no ICa.T is activated (Figure 4C). ICa.T reactivation τs averaged 51 ± 3 ms (control) versus 49 ± 4 ms (CHF, n = 10 cells/group, P = NS). The frequency dependence of ICa.T (Figure 4F) was greater than that of ICa.L but did not differ between groups.

Figure 7. AP results for control (left) and CHF (right) Purkinje fiber preparations. A and B, Typical AP recordings. Vertical line to the left of each AP shows phase-1 amplitude. P.V. indicates the point used to measure voltage at the onset of the plateau. C and D, Effects of E-4031 on APs at 1 Hz in control (C) and CHF (D) PCs. E and F, Effects of E-4031 on mean ± SEM APD in 20 control (E) and 26 CHF (F) PCs. *P < 0.05, **P < 0.01, ***P < 0.001 for difference between CHF and control PCs under corresponding conditions with respect to frequency and drug. CTL indicates control.
Kinetics. Recent studies point to downregulation of I_{Kr} in ventricular myocytes of rabbits with CHF.\(^5,18,19\) This has been studied to a lesser extent, but recent studies suggest that I_{Kr} also tends to be decreased by \(\approx 25\%\).\(^14\) Although there is some variability in the results for I_{Ca,L}, overall there seems to be no change in I_{Ca,L} density or kinetics. Recent studies point to downregulation of I_{Kr} and possibly I_{Ca,L} in ventricular myocytes of rabbits with tachypacing-induced CHF.\(^2,4\) Atrial ionic remodeling in CHF has been studied to a lesser extent, but recent studies suggest that I_{Kr} and I_{Ca,L} are all decreased and I_{NCX} is increased, with no change in kinetics or voltage dependence and no change in I_{K1} or I_{Ks}.\(^6\)

Several aspects of PC ionic remodeling resemble previously reported findings in ventricular myocytes: I_{in} and I_{K1} were reduced and I_{Ca,L} density was unchanged. These observations suggest that the mechanisms leading to CHF-induced decreases in I_{in} and I_{K1} downregulation at the ventricular level also likely operate on free-running Purkinje fiber false tendons. The CHF-induced slowing of I_{Ca,L} inactivation that we have observed has, to our knowledge, been reported in ventricular myocytes. We did not observe changes in I_{K1} or I_{Ks}, suggesting that PCs may be spared from the I_{K1} downregulation occurring with CHF in ventricular myocytes and possibly explaining why overall APD was not prolonged in CHF PCs. Unlike typical findings in ventricular and atrial myocytes, I_{NCX} density was not increased by CHF in PCs.

**Comparison with Previous Studies of CHF-Induced Ionic Remodeling**

There have been extensive studies of the ionic remodeling of K\(^+\) and Ca\(^{2+}\) channels in ventricular myocytes of patients and experimental animals with CHF.\(^5,18,19\) I_{in} is quite consistently reduced\(^5,20–22\) by an average of \(\approx 35\%\) in ventricular myocytes of patients with CHF.\(^18\) I_{K1} also tends to be decreased by \(\approx 25\%.\(^14\) Although there is some variability in the results for I_{Ca,L},\(^2,3\) overall there seems to be no change in I_{Ca,L} density or kinetics. Recent studies point to downregulation of I_{Kr} and possibly I_{Ca,L} in ventricular myocytes of rabbits with tachypacing-induced CHF.\(^2,4\) Atrial ionic remodeling in CHF has been studied to a lesser extent, but recent studies suggest that I_{in}, I_{Kr}, and I_{Ca,L} are all decreased and I_{NCX} is increased, with...
intercurrent diseases. Consistent with this notion, PCs from dogs with CHF showed twice as great APD prolongation in response to \( I_{Ks} \) blockade with E-4031 compared with control PCs. CHF significantly increases the risk of drug-induced Torsades de Pointes arrhythmias.\(^3\) The CHF-induced ionic remodeling that we observed in PCs could clearly play an important role in this clinically important phenomenon.

### Potential Limitations

The isolation of PCs from free-running Purkinje fibers is technically challenging, requiring prolonged periods of bath exposure to cell-dissociating enzymes (chunk method). This likely explains the absence of studies of PC remodeling in CHF, despite the large number of studies that have evaluated ventricular myocyte remodeling. \( I_K \) is particularly sensitive to isotype technique\(^26\) and has been difficult to record in isolated PCs.\(^12,22\)\(^<\) We were able to record robust \( I_K \) in isolated PCs, but \( I_{Ks} \) was smaller and more difficult to record; therefore, our results regarding \( I_{Ks} \) should be interpreted with caution. The properties of the currents we recorded from normal PCs were generally similar to those reported for PCs. CHF significantly increases the risk of drug-induced torsade de pointe with d,l-sotalol.\(^3\) The CHF-induced ionic remodeling that we observed in PCs could clearly play an important role in this clinically important phenomenon.

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