Ionic Remodeling of Cardiac Purkinje Cells by Congestive Heart Failure

Wei Han, MSc; Denis Chartier, BSc; Danshi Li, MD, PhD; Stanley Nattel, MD

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 tachyarrhythmias and the occurrence of sudden death. In addition, CHF promotes drug-induced Torsades de Pointes arrhythmias. Abnormal repolarization, related to ion channel remodeling, is important in the arrhythmogenic potential of CHF. CHF-induced remodeling of ionic currents in ventricular and atrial 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. Ionic currents are altered in subendocardial PCs in regions of myocardial infarction; 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 described 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 described.

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 IC₅₀ 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 (Iₚₔ) was studied as 5 μmol/L E4031-sensitive current and inward rectifier current (Iₖᵣ) as 1 mmol/L Ba²⁺-sensitive current. Slow delayed rectifier current (Iₚₛ)
was studied in the presence of 1 μmol/L dofetilide to eliminate \( I_{\text{Kf}} \).
For \( I_{\text{Kf}} \) 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 \( I_{\text{Kf}} \) 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, nimodipine 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 \( I_{\text{Kf}} \) exchange, \( Na^{+}\)-Ca\(^{2+}\) 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 constant \((τ)_c\) averaged 2.5±0.1 MΩ and 290±10 μs. Leakage compensation was not used. The capacitance of CHF cells was increased so currents are expressed in terms of density.

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

Figure 2A illustrates \( I_{\text{Kf}} \) recordings from control and CHF PCs. \( I_{\text{Kf}} \) density was significantly smaller in CHF PCs (Figure 2C); however, there were no differences in the form of the \( I_{\text{Kf}} \) vs voltage relation (Figure 2D). The voltage dependence of \( I_{\text{Kf}} \) inactivation was tested with a 2-pulse protocol as described in Figure 2E. Activation voltage dependence was evaluated from the relation \( I_{\text{Kf}} = a_{\text{Kf}}G_{\text{max}}(V_{TP}) \), where \( I_{\text{Kf}} \) vs voltage was plotted for each cell (control and CHF). The voltage dependence of \( I_{\text{Kf}} \) inactivation was determined by evaluating the \( \tau \) and \( V_{1/2} \) of the \( I_{\text{Kf}} \) vs voltage relation (Figure 2D). The voltage dependence of \( I_{\text{Kf}} \) inactivation was tested with a 2-pulse protocol as described in Figure 2E. Activation voltage dependence was evaluated from the relation \( I_{\text{Kf}} = a_{\text{Kf}}G_{\text{max}}(V_{TP}) \), where \( I_{\text{Kf}} \) vs voltage was plotted for each cell (control and CHF). The voltage dependence of \( I_{\text{Kf}} \) inactivation was determined by evaluating the \( \tau \) and \( V_{1/2} \) of the \( I_{\text{Kf}} \) vs voltage relation (Figure 2D).
(Figure 2G) was biexponential, with \( \tau \) averaging 42±8 and 139±67 ms (control) and 52±11 and 1486±108 ms (CHF, \( n = 10 \) for each, \( P = \text{NS} \)). \( I_{\text{Kr}} \) frequency dependence was similarly unaffected by CHF (Figure 2H).

Figure 3 shows results for \( I_{\text{Kr}} \) (left) and \( I_{\text{Ks}} \) (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 \( I_{\text{Kr}} \) or \( I_{\text{Ks}} \).

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

Representative L-type calcium current (\( I_{\text{Ca.L}} \)) recordings are shown in Figures 4A and 4B and point to slowed \( I_{\text{Ca.L}} \) decay in CHF. \( I_{\text{Ca.L}} \) 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, \( \tau_{\text{a}} \) increased from 6.0±0.3 to 8.2±0.9 ms (\( P < 0.05 \)) and \( \tau_{\text{inactivation}} \) 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, \( \tau_{\text{inactivation}} \) accounted for 36±2% of inactivation in control PCs compared with 51±5% in CHF (\( P < 0.05 \)). \( I_{\text{Ca.L}} \) inactivation voltage dependence was assessed with a 2-pulse protocol (Figure 4F). Inactivation \( \tau_{\text{a}} \) 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 \( I_{\text{V}} = \beta_{\text{phys}} G_{\text{max}} \) \((V_{\text{m}} - V_{\text{th}})\), with \( V_{\text{th}} \) obtained from a linear fit to the ascending portion of the \( I-V \) relation. Resulting mean \( \tau_{\text{a}} \) and slope factors were 2.0±1.4 and 6.6±0.2 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 monoexponential, with similar \( \tau \) in control (44±7 ms) and CHF PCs (53±6 ms, \( n = 10 \) group, \( P = \text{NS} \)). \( I_{\text{Ca.L}} \) showed little frequency dependence between 1 and 5 Hz at a HP of −80 mV, and there were no significant CHF-related differences in \( I_{\text{Ca.L}} \) frequency dependence (Figure 4H).
Ca.T reactivation was assessed (Figure 5E) at a test potential of +50 mV in the presence of CaCl₂. Reverse-mode NCX is substantial in the presence of 5 mmol/L CaCl₂ and is absent in the presence of 0 mmol/L CaCl₂, allowing NCX to be calculated from the difference between the two current recordings. The results in Figure 6B show that NCX 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 NCX amplitude was larger in CHF cells but that after correction for cell size (capacitance normalization), there were no significant differences.
plateau voltage was higher (Figure 7, top). There were no significant differences in resting potential, AP amplitude, or AP duration (APD) between control and CHF cells, but plateau-voltage was significantly more positive and phase 1 amplitude significantly smaller in CHF PCs (Table).

To evaluate the possibility that the response to an $I_{Kr}$- blocking class III drug may be altered in CHF PCs, APs were recorded from control and CHF PCs in free-running false tendons with standard microelectrodes before and after exposure to E-4031 (1 μmol/L). The results are illustrated in Figures 7C and 7D, and mean data are provided in Figures 7E and 7F. As in the first series of experiments, predrug APDs were not significantly different between control and CHF PCs. However, E-4031 had substantially larger effects on APD in CHF PCs, so that APD$_{50}$ after the drug was significantly greater in CHF cells at all frequencies (P<0.001 for each). For example, E-4031-induced APD$_{50}$ increases averaged 103±45 ms (25±10%) at 1 Hz in control preparations compared with 231±40 ms (54±10%) in CHF preparations.

**Discussion**

We have evaluated the effects of CHF on $K^+$ currents, $Ca^{2+}$ currents, NCX, and AP properties of PCs from free-running ventricular false tendons. CHF decreased PC $I_{Na}$ and $I_{K1}$ density and slowed the inactivation of $I_{Ca,L}$ without altering its density. $I_{Ks}$, $I_{Kr}$, $I_{NCX}$, and $I_{Ca,T}$ were unaffected. CHF reduced the amplitude of phase 1 repolarization, increased plateau voltage, and enhanced the APD-prolonging effect of E-4031.

**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. $I_{Na}$ and $I_{K1}$ are quite consistently reduced by an average of ≈35% in ventricular myocytes of patients with CHF. $I_{Kr}$ also tends to be decreased by ≈25%. 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 $I_{Ca,L}$ and possibly $I_{Ca}$ in ventricular myocytes of rabbits with tachypacing-induced CHF. Atrial ionic remodeling in CHF has been studied to a lesser extent, but recent studies suggest that $I_{Na}$, $I_{Kr}$, and $I_{Ca}$ are all decreased and $I_{NCX}$ is increased, with no change in kinetics or voltage dependence and no change in $I_{K1}$ or $I_{Ks}$. Several aspects of PC ionic remodeling resemble previously reported findings in ventricular myocytes: $I_{Na}$ and $I_{K1}$ were reduced and $I_{Ca,L}$ density was unchanged. These observations suggest that the mechanisms leading to CHF-induced $I_{Na}$ 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 $I_{Ks}$ or $I_{Kr}$, suggesting that PCs may be spared from the $I_{Kr}$ 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.

**Relationship to Other Studies in PCs**

$I_{Ca,L}$, $I_{Ca,T}$, and E-4031–sensitive current have been studied in PCs from the subendocardial Purkinje fiber network overlaying 24- to 48-hour-old myocardial infarctions. $I_{Na}$ density was reduced by >50% in the infarct zone, with no change in voltage dependence but a slowing in reactivation. PCs from free-running false tendons had normal $I_{K1}$ properties. Both $I_{Ca,L}$ and $I_{Ca,T}$ were reduced in subendocardial PCs from the infarct zone, with no changes in voltage dependence or inactivation kinetics. $I_{K1}$ was reduced in PCs from the infarct zone, and a very rapidly activating E-4031–sensitive current was increased, with no classical $I_{Ks}$ noted in either normal or infarct zone PCs. In general, the abnormalities noted in infarct zone PCs were more severe than those we observed. There are, however, some qualitative similarities in terms of decreases in $I_{K1}$ and $I_{Na}$. In contrast to our findings, myocardial infarction did not affect the electrophysiology in PCs from free-running false tendons, suggesting that myocardial infarction produces severe but localized ionic remodeling in infarct zone PCs whereas CHF produces more generalized but less severe remodeling.

**Potential Significance**

The present study constitutes the first detailed analysis of CHF-induced ionic remodeling in PCs. PCs are believed to play an important role in ventricular arrhythmogenesis, particularly in the generation of early afterdepolarizations in patients with CHF, particularly in response to interventions such as $I_{Na}$-blocking antiarrhythmic drugs and hypokalemia that prolong APD. Downregulation of $I_{Na}$, along with slowed inactivation of $I_{Ca,L}$, is likely responsible for the positive shift in the plateau voltage of PCs. The positive shift in plateau voltage and slowed $I_{Ca,L}$ inactivation at positive voltages would act to promote the occurrence of $I_{Ca,L}$-dependent early afterdepolarizations under conditions that delay repolarization.

The CHF-induced decreases in $I_{Na}$ and $I_{K1}$ are likely to reduce the repolarization reserve, the ability of myocardial cells to repolarize when normal repolarizing currents are reduced by drugs, metabolic or electrolyte imbalances, or
intercurrent diseases. Consistent with this notion, PCs from dogs with CHF showed twice as great APD prolongation in response to \(I_{Kr}\) blockade with E-4031 compared with control PCs. CHF significantly increases the risk of drug-induced torsades de pointes arrhythmias. 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 isolation technique and has been difficult to record in isolated PCs. We were able to record robust \(I_{K}\) in isolated PCs, but \(I_{Kr}\) was smaller and more difficult to record; therefore, our results regarding \(I_{Kr}\) should be interpreted with caution. The properties of the currents we recorded from ventricular myocytes, removing an important role in this clinically important phenomenon.

**Acknowledgments**

This work was supported by operating grants from the Canadian Institutes for Health Research and the Quebec Heart and Stroke Foundation. Dr Li was a Heart and Stroke Corporation of Canada/Institutes for Health Research and the Quebec Heart and Stroke Foundation. The authors thank Essai and Pfizer Pharmaceuticals for technical assistance, and Annie Laprade for secretarial help.

**References**


Ionic Remodeling of Cardiac Purkinje Cells by Congestive Heart Failure
Wei Han, Denis Chartier, Danshi Li and Stanley Nattel

Circulation. 2001;104:2095-2100
doi: 10.1161/hc4201.097134

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/17/2095

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at: http://circ.ahajournals.org//subscriptions/