Ionic Remodeling of Sinoatrial Node Cells by Heart Failure

Arie O. Verkerk, PhD; Ronald Wilders, PhD; Ruben Coronel, MD, PhD; Jan H. Ravesloot, PhD; E. Etienne Verheijck, PhD

Background—In animal models of heart failure (HF), heart rate decreases as a result of an increase in intrinsic cycle length of the sinoatrial node (SAN). In this study, we evaluate the HF-induced remodeling of membrane potentials and currents in SAN cells.

Methods and Results—SAN cells were isolated from control rabbits and rabbits with volume and pressure overload-induced HF and patch-clamped to measure their electrophysiological properties. HF cells were not hypertrophied (capacitance, mean±SEM, 52±3 versus 50±4 pF in control). HF increased intrinsic cycle length by 15% and decreased diastolic depolarization rate by 30%, whereas other action potential parameters were unaltered. In HF, the hyperpolarization-activated “pacemaker” current ($I_f$) and slow component of the delayed rectifier current ($I_{ks}$) were reduced by 40% and 20%, respectively, without changes in voltage dependence or kinetics. T-type and L-type calcium current, rapid and ultrarapid delayed rectifier current, transient outward currents, and sodium-calcium exchange current were unaltered.

Conclusions—In single SAN cells of rabbits with HF, intrinsic cycle length is increased as the result of a decreased diastolic depolarization rate rather than a change in action potential duration. HF reduced both $I_f$ and $I_{ks}$ density. Since $I_{ks}$ plays a limited role in pacemaker activity, the HF-induced decrease in heart rate is attributable to remodeling of $I_f$.

Key Words: sinoatrial node ■ remodeling ■ ion channels ■ action potentials

Heart failure (HF) predisposes to life-threatening ventricular arrhythmias and sudden death. Abnormal repolarization, related to ion channel remodeling, is important in the arrhythmogenic potential of HF, especially at low heart rates. Clinically, patients with HF have a slower intrinsic heart rate. In animal models, HF decreases heart rate by increasing the intrinsic cycle length of the sinoatrial node (SAN), with a larger responsiveness to acetylcholine and a decreased circular rhythm. HF-induced remodeling of ionic currents has been studied in ventricular, atrial, and Purkinje myocytes. HF decreased transient outward K+ current ($I_{to}$) density in all three cell types, decreased slow component of the delayed rectifier current ($I_{ks}$) density in ventricular and atrial myocytes, and decreased L-type Ca2+ current ($I_{ca}$) density in atrial cells. However, little is known about the effects of HF on SAN pacemaker activity. In this study, we evaluate HF-induced remodeling of membrane potentials and currents in rabbit SAN cells.

Methods

Cell Preparation
Cardiac failure was induced in New Zealand White male rabbits (Enki, Kerkendijk, The Netherlands) by combined volume and pressure overload as described previously. Hearts were excised 3 to 4 months after severing the aortic valve and ligation of the abdominal aorta and the HF-index, based on relative heart weight, relative lung weight, left ventricular end-diastolic pressure, third heart sound, and ascites was calculated. We performed experiments if at least 3 of the 5 parameters were abnormal, thus indicating severe HF. Age-matched healthy animals served as control animals. Animal use followed institutional guidelines.

Single SAN cells were enzymatically isolated as described previously. Cells were allowed to adhere for 5 minutes, after which superfusion with Tyrode’s solution (36±0.5°C) was started. Spindle and elongated spindle-like cells displaying regular contractions were selected for electrophysiological measurements. Standard Tyrode’s solution contained (mmol/L): NaCl 140, KCl 5.4, CaCl2 1.8, MgCl2 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NaOH).

Data Acquisition and Analysis
Membrane potentials and currents were recorded with the use of the amphotericin-perforated or ruptured patch-clamp technique. Standard pipette solution contained (mmol/L) K-glucosate 125, KCl 20, HEPES 10, with or without amphotericin-B 2.2; pH 7.2 (KOH). Potentials were corrected for liquid junction potential. Signals were low-pass filtered (1-kHz cutoff frequency) and digitized at 2 kHz.

Action potentials were recorded with the use of the amphotericin-perforated patch-clamp technique and characterized by action potential duration (APD) at 20%, 50%, and 100% repolarization (APD20, APD50, and APD100, respectively), maximal diastolic potential...
Net membrane current was recorded with the use of the amphotericin-perforated patch-clamp technique and examined by 500-ms voltage-clamp steps from a holding potential (HP) of −40 mV every 2 seconds. The Ca\(^{2+}\)-activated Cl\(^{-}\) current (I_{SCX}) was recorded by use of the amphotericin-perforated patch-clamp technique and examined by 500-ms depolarizing voltage-clamp steps (HP −40 mV; every 2 seconds). The I_{SCX} was defined as the transient outward current sensitive to 0.2 mmol/L 4,4′-disothiocyanatostilbene-2,2′-disulfonic acid (DIDS).

Detailed measurements of calcium current (I_{Ca}), I_{Ks}, I_{Kr}, and Na\(^+\)-Ca\(^{2+}\) exchange current (I_{SCX}) were performed by use of the ruptured patch-clamp technique, with 10 mmol/L EGTA added to the pipette solution, except for I_{SCX} measurements. For I_{ES} measurements, CsCl replaced all K-glucurate and KCi in the pipette solution. 7,8 TEA-Cl and CsCl replaced NaCl and KCl, respectively, in the Tyrode’s solution. 1,3 I_{CaL} was measured in the presence of 0.5 mmol/L CdCl\(_2\), as transient outward current sensitive to 10 mmol/L 4-aminoypyridine (4AP). 3,13 I_{Ks} was activated by a 2-step voltage-clamp protocol (HP −40 mV; every 5 seconds; for example, see Figure 4A).

For detailed measurements of ultrarapid, rapid, and slow components of the delayed rectifier current (I_{K1}, I_{K2}, and I_{K3}, respectively), Tyrode’s solution contained 5 μmol/L nifedipine to block I_{CaL}. I_{K} components were measured in the same cell by 4-second depolarizing pulses (HP, −40 mV; every 10 seconds). First, I_{K} was measured as 5 μmol/L E4031-sensitive current. 13 Next, I_{K} was studied in the continued presence of E4031 as 1 mmol/L 4AP-sensitive current. Finally, I_{K} was studied in the continued presence of E4031 and 4AP as the remaining time-dependent current. 13

I_{f} was examined as time-dependent current during 2-second hyperpolarizing voltage-clamp steps (HP, −40 mV; every 4 seconds). 14 For determination of I_{f} tail currents, Tyrode’s solution contained 5 μmol/L nifedipine and 40 μmol/L NiCl\(_2\) to block I_{CaL} and T-type calcium current (I_{CaT}), respectively.

I_{SCX} was measured as 5 mmol/L Ni\(^{2+}\)-sensitive current during a ramp protocol and conditions, as described previously in detail. 13 For these experiments, pipette solution contained (mmol/L): CsCl 145, NaCl 5, Mg\(^{2+}\)-ATP 10, TEA-Cl 20, HEPES (NMDG-OH) 10, EGTA 20, CaCl\(_2\) 20 (calculated free Ca\(^{2+}\) = 150 mmol/L); pH 7.2 (NMDG-OH). To suppress membrane currents other than I_{SCX}, the following blockers were added to a K\(^{-}\)-free Tyrode’s solution (mmol/L): BaCl\(_2\), 1; CsCl 2, nifedipine 0.005, ouabain 0.1, DIDS 0.2. A ramp pulse from −100 to +50 mV and then back to −100 mV was given every 18 seconds. The I_{SCX} I-V relation was obtained from the descending phase.

I_{K1}, I_{K2}, and I_{f} activation curves were obtained by plotting normalized tail current amplitude against potential. Boltzmann fits were used to determine V_{1/2} (membrane potential for half-maximal activation) and k (slope factor). I_{f}, I_{K1}, and I_{f} deactivation kinetics were analyzed using biexponential fits, ignoring the variable initial delay in I_{f} (de)activation. 12,13 All drugs were obtained from Sigma except E4031, which was a gift from Eisai.

**Statistics**

Results are expressed as mean±SEM. Two sets of data were considered significantly different if the probability value of the unpaired Student’s t-test with Bonferroni correction was <0.05.

**Results**

**HF Reduces Intrinsic Firing Rate**

Representative action potentials from a control and an HF cell are shown in Figure 1A. The HF cell has a lower DDR and a lower intrinsic firing rate. Figure 1B summarizes action potential characteristics of control and HF cells. HF cells display a 30% decrease in DDR and a 15% increase in intrinsic CL without significant differences in MDP, action potential overshoot, or action potential duration. Membrane capacitance was similar in control and HF cells (Figure 1B), indicating absence of hypertrophy of HF cells. These data indicate that HF cells have a reduced intrinsic firing rate due to a reduced DDR.

**HF Reduces Net Membrane Current During Hyperpolarization**

Cells used for action potential recordings were also subjected to generic voltage-clamp protocols, without specific channel blockers, to evaluate (net) membrane currents that could underlie the observed differences in action potential config-
uration. Under these conditions, several currents activate simultaneously. Therefore, we only analyzed the net membrane current at the beginning (\(I_{begin}\)) and end (\(I_{end}\)) of depolarizing (Figure 1C) and hyperpolarizing (Figure 1D) voltage-clamp steps. The resulting current-voltage (I-V) relations (Figure 1E) only show a significant reduction in steady-state currents in the voltage range where \(I_f\) is active (asterisks).

**HF Reduces \(I_f\) and \(I_{Ks}\) Density**

Our current-clamp data demonstrate that HF cells have a reduced DDR and, accordingly, a reduced intrinsic firing rate. The voltage-clamp data indicate that this is due to a decrease in net membrane current during hyperpolarization. To evaluate the effects of HF on individual membrane currents, we next carried out voltage-clamp experiments with specific blockers or solutions.

**Calcium Currents**

A representative series of \(I_{Ca}\) traces recorded in control and HF cells is shown in Figure 2A. The depolarizing steps elicit time- and voltage-dependent inward currents typical of \(I_{Ca}\). \(I_{Ca,L}\) was measured as \(I_{Ca}\) elicited at HP of −50 mV.\(^9\) \(I_{Ca,T}\) was obtained by digital subtraction of \(I_{Ca}\) traces elicited stepping from HPs of −50 and −90 mV.\(^9\) \(I_{Ca,T}\) was not present in all cells, consistent with our previous findings.\(^8\) \(I_{Ca,T}\) was found in 40% and 43% of control (n=15) and HF (n=14) cells, respectively. Neither amplitude nor shape of the \(I_{Ca,L}\) and \(I_{Ca,T}\) I-V relations were altered by HF (Figure 2B).

**Transient Outward Currents**

The presence of transient outward currents in SAN cells is not a consistent finding. The DIDS-sensitive transient outward current, \(I_{Cl(Ca)}\), is present in only 33% of rabbit SAN cells.\(^10\) Moreover, some investigators describe a large 4AP-sensitive transient outward current, \(I_{to1}\), whereas others report little or no \(I_{to1}\) (see Reference 16 and primary references therein).\(^16\) Figure 3A shows superimposed current traces recorded in the absence and presence of 0.2 mmol/L DIDS. In the absence of

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**Figure 2.** Heart failure does not alter L-type and T-type Ca\(^{2+}\) currents (\(I_{Ca,L}\) and \(I_{Ca,T}\)). A, Current traces elicited by depolarizing voltage steps from −90 mV to +50 mV. \(I_{Ca,T}\), if present, was obtained as the difference current. B, Average I-V relations of \(I_{Ca,T}\) and \(I_{Ca,L}\).

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**Figure 3.** Heart failure does not alter Ca\(^{2+}\)-activated Cl\(^-\) current (\(I_{Cl(Ca)}\)). A, Current traces elicited by depolarizing voltage steps in absence and presence of 0.2 mmol/L DIDS. B, DIDS-sensitive current (\(I_{Cl(Ca)}\)). C, Average I-V relation of \(I_{Cl(Ca)}\).
DIDS, the traces showed a transient outwardly directed current component, which was blocked by DIDS. By digitally subtracting the two traces, the DIDS-sensitive $I_{Cl(Ca)}$ was obtained (Figure 3B). $I_{Cl(Ca)}$ was found in 42% and 36% of control (n=14) and HF (n=14) cells, respectively, without any difference in the I-V relation (Figure 3C). No DIDS-sensitive steady-state currents were observed (Figure 3B), indicating that HF does not induce a swell-activated Cl- current as previously observed in dog and rat but not rabbit ventricular cells (see Reference 17 and primary references therein).17

In all cells tested, we were unable to measure a substantial $I_{to1}$ (Figure 4). Figure 4B shows superimposed current traces recorded in the absence and presence of 10 mmol/L 4AP. The preconditioning hyperpolarizing step elicits the time- and voltage-dependent inward current typical of $I_f$ (and thus smaller in the HF cells; compare Figures 1 and 6). The subsequent depolarization steps elicit a transient outward current (“$I_{to}$”), which is due to the decaying tail current of $I_f$ ($I_{f,tail}$), and, if present, also $I_{to1}$ activation and subsequent inactivation (Figure 4A). 4AP significantly reduced steady-state activation of $I_f$ ($I_{f,ss}$) as well as $I_{to}$ (Figure 4, B and C). In both control and HF cells, the $I_{f,ss}$ decrease is similar to the $I_{to}$ decrease (Figure 4D), indicating that $I_{f,tail}$ is the main component of “$I_{to}$.” This is confirmed by calculating the control $I_{f,tail}$ through the formula $I_{f,tail}=I_{f,0}^{4AP}/I_{f,0}^{control}$ and subtracting this from the control “$I_{to}$,” thus obtaining $I_{to1}$. The calculated, small, $I_{to1}$ amplitude did not differ between control and HF cells (Figure 4C).

**Delayed Rectifier Currents**

Superimposed current traces were recorded under control conditions, in the presence of E4031, and in the combined presence of E4031 and 4AP (Figure 5A) to discriminate between $I_{Kr}$, $I_{Kur}$, and $I_{Ks}$ (Figure 5B). No changes in $I_{Kr}$ amplitude (Figure 5C) or kinetics (Figure 5D) were observed. Also, no changes were observed in the I-V relation of $I_{Kur}$ (Figure 5E). Activation of $I_{Kur}$ was practically instantaneous (activation kinetics could not be resolved) without detectable tail currents on repolarization (Figure 5B). Both the step and tail density of $I_{Ks}$ were reduced by 20% by HF (Figure 5F) without changes in kinetics (Figure 5G).

Figure 4. Heart failure does not alter transient outward K- current ($I_{to}$). A, Diagram of voltage-clamp protocol (left) and activated current components (right), for example, steady-state hyperpolarization-activated current ($I_{f,ss}$) and transient outward current (“$I_{to}$,” comprising $I_{to1}$ and $I_{f,tail}$). B, Current traces elicited in absence and presence of 10 mmol/L 4AP. C, Average amplitude of $I_{f,ss}$ and “$I_{to}$” in absence and presence of 4AP. D, Normalized effects of 4AP on $I_{f,ss}$ and $I_{to}$.

Figure 5. Heart failure does not alter rapid and ultrarapid delayed rectifier current ($I_{Kr}$ and $I_{Kur}$) but reduces slow delayed rectifier current ($I_{Ks}$). A, Current traces elicited by depolarizing voltage steps under control conditions, in presence of 5 μmol/L E4031 and 4AP (Figure 5A) to discriminate between $I_{Kr}$, $I_{Kur}$, and $I_{Ks}$ (Figure 5B). No changes in $I_{Kr}$ amplitude (Figure 5C) or kinetics (Figure 5D) were observed. Also, no changes were observed in the I-V relation of $I_{Kur}$ (Figure 5E). Activation of $I_{Kur}$ was practically instantaneous (activation kinetics could not be resolved) without detectable tail currents on repolarization (Figure 5B). Both the step and tail density of $I_{Ks}$ were reduced by 20% by HF (Figure 5F) without changes in kinetics (Figure 5G).
Hyperpolarization-Activated Current
A representative series of $I_f$ traces recorded in control and HF cells is shown in Figure 6A. Both the step and tail amplitude of $I_f$ were reduced by HF (Figure 6B). Over the entire potential range studied, $I_f$ amplitude was 40% lower in HF compared with control cells, without any changes in kinetics (Figure 6C).

Na$^+$-Ca$^{2+}$ Exchange Current
Representative current traces during ramp hyperpolarizations in the absence and presence of Ni$^{2+}$ are shown in Figure 7A. $I_{NCX}$ was measured as the Ni$^{2+}$-sensitive current. The associated $I$-$V$ relation of $I_{NCX}$ was not altered by HF (Figure 7B).

Computer Simulations
We found that HF specifically reduced $I_f$ by 40% and $I_{Ks}$ by 20% in SAN cells. To assess whether these reductions may be responsible for the increase in intrinsic CL, we carried out computer simulations with the use of comprehensive mathematical models of a single rabbit SAN cell (Figure 8). The effects of the reduction in $I_{Ks}$ are negligible (dashed lines). The increase in intrinsic CL—by 3.4% and 5.9% in the central and peripheral SAN model cell, respectively—is due to the decrease in DDR on $I_f$ reduction (solid lines).

Discussion
In SAN cells, pacemaker activity is due to a complex interplay of many time- and voltage-dependent currents (see Reference 19 and primary references therein). In the current

Figure 6. Heart failure reduces hyperpolarization-activated current ($I_f$). A, Current traces elicited by hyperpolarizing voltage steps. B and C, Average $I$-$V$ relation (B) and (de)activation kinetics (C) of $I_f$.

Figure 7. Heart failure does not alter Na$^+$-Ca$^{2+}$ exchange current ($I_{NCX}$). A, Current traces elicited by ramp protocol in absence and presence of 5 mmol/L NiCl$_2$ and the Ni-sensitive current ($I_{NCX}$). B, Average $I$-$V$ relations of $I_{NCX}$.

Figure 8. Effects of reduction in $I_f$ (by 40%) and $I_{Ks}$ (by 20%) density in Zhang et al central (A) and peripheral (B) rabbit SAN cell model.
study, we demonstrate that HF increased intrinsic CL, caused by a decrease in DDR rather than changes in APD (Figure 1). Voltage-clamp experiments demonstrated that HF reduced $I_f$ and $I_{Ks}$ density by 40% and 20%, respectively (Figures 5 and 6), without affecting other membrane currents, including $I_{CaL}$, $I_{CaT}$, $I_{Kur}$, $I_{Ks}$, $I_{HCN}$, and $I_{NCX}$ (Figures 2 to 5 and 7). Considering the important role of $I_f$ in setting DDR and, consequently, CL,$^{13,14}$ the reduced $I_f$ density provides a plausible explanation for the increased intrinsic CL, which was supported by our computer simulations (Figure 8). These simulations also show that the effects of the $I_{Ks}$ reduction are minimal, in agreement with recent experimental findings showing a negligible role of $I_{Ks}$ in setting pacemaker rate in rabbit without β-adrenergic stimulation.$^{20}$

**HF Reduces $I_f$ and $I_{Ks}$ Density**

Our experiments show that $I_f$ density is smaller in rabbit SAN cells isolated from failing hearts. In rabbit SAN cells, a morphology-dependent variation in $I_f$ density is found, with a smaller $I_f$ in spindle-like compared with spider-like cells.$^{21}$ Since we only used spindle-like cells, we can exclude a possible role of cell morphology in our experiments. Moreover, a cell size–dependent variation in the density of various cation currents, including $I_f$, $I_{CaT}$, and $I_{Ks}$, is found.$^{22}$ We exclude cell size–dependency as an explanation for our principal findings because cell capacitance did not differ between control and HF (Figure 1). Finally, an age-related variation in $I_f$ density was found, with a smaller $I_f$ in SAN cells isolated from adult compared with newborn rabbit.$^{23}$ We also exclude age dependency because control and HF rabbits were age-matched.

In rabbit SAN cells, we found that HF reduced $I_f$ density, which contrasts with findings in human ventricular cells isolated from end-stage failing hearts, in which $I_f$ density was unchanged$^{24}$ or increased.$^{25}$ The discrepancy may be due to a species dependency, but differences in functional properties of $I_f$ between SAN and ventricles may also play a role. Molecular characterization of $I_f$ has demonstrated that the Hyperpolarization-activated Cyclic Nucleotide gated (HCN) family$^{26}$ comprises four members, HCN1 to HCN4. All except HCN3 are present in heart, and their relative message levels vary with region and age.$^{27}$ The SAN largely contains isoforms HCN1 and HCN4. Ventricle largely contains isoforms HCN2 and HCN4, with a larger HCN2/HCN4 mRNA ratio in adult than newborn.$^{27}$ It is tempting to speculate that the presence of different isoforms, with distinct functional properties (Reference 28 and primary references therein),$^{28}$ is responsible for the different findings in rabbit SAN cells and human ventricular cells.

In rabbit SAN cells, we found that HF reduced $I_{Ks}$ density, which is consistent with findings in atrial and ventricular cells,$^{6,7}$ but contrasts with findings in Purkinje cells.$^8$ We found an unchanged $I_{CaL}$ in SAN cells, which agrees with findings in ventricular and Purkinje cells,$^7,8$ but contrasts with findings in atrial cells in which $I_{CaL}$ was reduced.$^7$ In our SAN cells, $I_{CaT}$, $I_{Kur}$, and $I_{Ks}$ were not altered by HF, as in atrial and Purkinje cells.$^7,8$ Whether $I_{Kur}$, defined in our experiments as 4AP-sensitive current activated from a HP of $–40$ mV, is indeed carried by Kv1.5 channels as found in guinea pig and ferret SAN$^{29}$ or is carried by other K$^+$ current components is not completely clear. $I_{HCN}$ was unaltered by HF, which agrees with findings in ventricular cells.$^{30}$ In rabbit SAN cells, we found that HF does not alter $I_{NCX}$ density, which is consistent with findings in Purkinje cells,$^8$ but contrasts with findings in atrial cells in which $I_{NCX}$ was increased.$^7$

In atrial, Purkinje, and ventricular cells, $I_{Ks}$ density is decreased in HF, which plays an important role in the observed action potential prolongation in these cell types.$^{7,8}$ As set out above, the presence of $I_{Ks}$ in SAN cells is not a consistent finding. When present, blockade of $I_{Ks}$ with 4AP increases APD and CL and decreases action potential overshoot, DDR, and MDP.$^{11}$ In our current-clamp experiments, we observed a decrease only in CL (Figure 1), suggesting that $I_{Ks}$ was not changed by heart failure or not functionally present. This was confirmed in our voltage-clamp experiments (Figure 4).

Altogether, the effects of HF on $I_{NCX}$, Ca$^{2+}$, and K$^+$ currents of SAN cells are comparable with findings in atrial, Purkinje, and ventricle, although some discrepancies exist. These discrepancies may be due to HF model, species, or tissue dependencies, but methodology differences may also play a role. Nevertheless, the absence of HF effects on $I_{NCX}$, Ca$^{2+}$, and K$^+$ currents agrees with our findings of unaltered APD.

**Computer Simulations**

Our simulation results of the effects of HF on intrinsic pacemaker activity of SAN cells (Figure 8) are qualitatively similar to the experimentally observed effects and thereby support our experimental findings. There are, however, quantitative differences (compare Figures 1 and 8), which may reflect the inherent limitation of SAN cell models to quantitatively reproduce the effects of $I_f$ block. In experiments on isolated rabbit SAN cells, block of $I_f$ by Cs$^+$ induced a 41% increase in cycle length,$^{11}$ whereas a simulated complete block of $I_f$ increases cycle length by 10% and 30% in the Zhang et al central and peripheral cell models,$^{10}$ respectively. Also, it is possible that HF alters intracellular calcium transients, which may affect calcium-sensitive currents such that DDR is further decreased.

**Conclusions**

In single SAN cells from rabbits with HF, an increase in intrinsic CL occurs due to a decreased DDR rather than changes in APD. The decrease in DDR can be attributed to remodeling of $I_f$, which resulted in a decreased $I_f$ density.

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