Chronic heart failure and the substrate for atrial fibrillation

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1. Introduction

Heart failure (HF) is a chronic irreversible syndrome and develops over months to years. HF is an increasingly prevalent disease and continues to result in substantial morbidity and mortality.¹,² A common co-morbidity during HF is atrial fibrillation (AF); HF increases the risk of developing AF by approximately four- to six-fold.³,⁴ Significantly, the development of AF in patients with advanced HF predicts a significantly increased risk of death.⁵,⁶

Although it has long been known that chronic HF increases the incidence of AF,⁶ the underlying causal mechanisms have not been well defined. Previous studies have evaluated HF-induced atrial remodelling after 2–6 weeks of HF induced by right ventricular (RV) tachypacing,⁶–⁸ and reported increased interstitial fibrosis, although myocytes from the right atrium had action potential (AP) prolongation accompanied by a modest decrement in I<sub>Ca,L</sub>, with reductions in I<sub>Na</sub> and I<sub>Ks</sub>.⁶,⁷ Inducible AF of seconds to minutes in duration occurs after this duration of HF.⁵,⁷

To examine the mechanisms whereby chronic HF induces a substrate for AF, we used a modification of a previously validated model of long-term, irreversible HF.⁹ Our data indicate that during chronic HF, atrial electrophysiological remodelling is distinct from that previously reported after 2–6 weeks of RV tachypacing. We report that this model results in a substrate for sustained AF, is accompanied by attenuation of atrial refractory period, and is attributable to a specific constellation of ion current abnormalities and shortening of the atrial AP duration (APD).

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K-channel

Aims We sought to define the underlying mechanisms for atrial fibrillation (AF) during chronic heart failure (HF).

Methods and results Preliminary studies showed that 4 months of HF resulted in irreversible systolic dysfunction (n = 9) and a substrate for sustained inducible AF (> 3 months, n = 3). We used a chronic (4-month) canine model of tachypacing-induced HF (n = 10) to assess atrial electrophysiological remodelling, relative to controls (n = 5). Left ventricular fractional shortening was reduced from 37.2 ± 0.83 to 13.44 ± 2.63% (P < 0.05). Left atrial (LA) contractility (fractional area change) was reduced from 34.9 ± 7.9 to 27.9 ± 4.23% (P < 0.05). Action potential durations (APDs) at 50 and 90% repolarization were shortened by ~60 and 40%, respectively, during HF (P < 0.05). HF-induced atrial remodelling included increased fibrosis, increased I<sub>Ca,L</sub> and decreased I<sub>Kr</sub>, I<sub>Kur</sub> and I<sub>Ks</sub> (P < 0.05). HF induced increases in LA Kv channel interacting protein 2 (P < 0.05), no change in Kv4.3, Kv1.5, or Kir2.3, and reduced Kir2.1 (P < 0.05). When I<sub>Ca,L</sub> was elicited by action potential (AP) clamp, HF APs reduced the integral of I<sub>Ca,L</sub> in control myocytes, with a larger reduction in HF myocytes (P < 0.05). I<sub>Ca,L</sub> measured with standard voltage clamp was unchanged by HF. Incubation of myocytes with N-acetylcycteine (a glutathione precursor) attenuated HF-induced electrophysiological alterations. LA angiotensin-1 receptor expression was increased in HF.

Conclusion Chronic HF causes alterations in ion channel expression and ion currents, resulting in attenuation of the APD and atrial contractility and a substrate for persistent AF.
2. Methods

All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). A total of 22 hound type dogs of either sex (2–3 years of age) had an RV pacemaker lead implanted in the RV apex as described previously. Following recovery from the pacemaker implant, the RV was paced at 180 bpm for 2 weeks; 200 bpm for the next 6 weeks, followed by 180 bpm for the duration of the protocol (modified Preveil 8086 pacemakers, Medtronic, Inc., Minneapolis, MN, USA). Sequential electrocardiograms, two-dimensional (2D) and M-mode echocardiograms, were performed at baseline during butorphanol sedation (0.5 mg/kg, im).10

2.1 Pilot studies

Twelve dogs were used for in vivo pilot studies to assess reversibility of HF and inducibility of persistent AF. HF was induced as described above with a duration of 16–17 weeks of RV tachypacing. After 16–17 weeks of RV tachypacing, one subset (n = 9) had pacing terminated to assess reversibility of HF. A second subset (n = 3) was used for a pilot study (n = 3) to evaluate atrial arrhythmia inducibility using premature extrastimuli after 16 weeks of RV tachypacing; in this subset, persistent AF was induced in each animal. After AF induction, the RV pacemaker rate was reduced to 30 bpm, heart rhythm was assessed by electrocardiography daily for a week, and weekly thereafter for up to 3 months; ventricular rates were ~140–170 bpm during AF.

2.2 Main study

On the basis of the results of the pilot studies, a group of RV tachypaced dogs (n = 10) were prepared to further assess mechanisms of atrial electrophysiological remodelling during chronic irreversible HF, using a duration (>16 weeks) when the pilot studies indicated the formation of a substrate for persistent AF. Atrial electrophysiology was assessed in vivo and in isolated left atrial (LA) appendage myocytes. Five age-matched dogs served as controls for these studies. Atrial effective refractory periods (ERPs) and AF inducibility were assessed as described previously.10 To measure atrial contractility non-invasively, we adapted established methods to the canine LA.11,12 Images were obtained in the 2D parasternal long-axis view, and % fractional area change (FAC) was calculated as FAC = (LA area end-systole – LA area end-diastole)/LA area end-systole × 100. Hemodynamic assessments were conducted in the main study just prior to euthanasia to evaluate end-diastolic pressure and peak left ventricular (LV) systolic pressure. Animals were pre-anaesthetized with butorphanol (0.4 mg/kg iv). Anaesthesia was sustained with isoflurane (1–1.5% with 100% oxygen). Body temperature was kept constant (37.0 ± 0.5 °C) with a water-heated blanket. A 5 Fr catheter-tip manometers (model SCP-350, Millar Instruments Inc.) were inserted to the LV via carotid artery under the fluoroscopic guidance to obtain peak LV pressure (LV P) and end-diastolic pressure (EDP) in mmHg. ECG lead II was also obtained. All recordings were made on a physiological data acquisition system (MP100WSW, Biopac Systems, Inc., Santa Barbara, CA, USA), and measurements were obtained from the average of 10 consecutive beats.

After completion of the in vivo measurements, dogs were anaestheitized by intravenous injection of pentobarbitonal sodium;13 cardiac tissue was obtained from the LA for protein analyses, histology, and myocyte isolation. LA appendage myocytes were isolated using a modification of previously described methods16 by cannulation and perfusion of the left circumflex artery. After completion of the previously described method of perfusion with collagenase, the LA appendage was minced and placed into 5 mL of perfusate in a shaking water bath at 37 °C for 5–15 min. Atrial myocytes were filtered and then centrifuged twice with incubation buffer (IB) to remove collagenase, prior to suspension in storage buffer. The myocytes were stored at room temperature in a standard IB solution containing (in mM): 118 NaCl, 4.8 KCl, 1.2 MgCl2, 1.2 KH2PO4, 0.68 glutamine, 10 glucose, 5 pyruvate, 1 CaCl2, along with 1 µmol/L insulin and 1% BSA until used. This typically yielded 40–60% rod-shaped myocytes with staircase ends and sharp margins. In some experiments, myocytes were stored in IB containing 10 mM N-acetylcysteine (NAC), a membrane-permeable precursor of glutathione. All myocyte experiments were conducted within 10 h of isolation.

2.3 Electrophysiological protocols

Data acquisition was performed with Clampex version 8.0 or 9.0 software (Axon Instruments, Union City, CA, USA) and Axopatch 200A patch clamp amplifiers (Axon Instruments Inc.). Perforated whole-cell patch-clamp (using amphoterin B) was used to minimize alterations in intracellular milieu during AP, AP clamp, and potassium current recordings. Myocytes were placed in a laminin-coated cell chamber (Cell Microcontrols, Norfolk, VA, USA) and superfused with solution containing (in mM): 135 NaCl, 5 MgCl2, 5 KCl, 10 glucose, 1 CaCl2, 5 HEPES, pH adjusted to 7.40 with NaOH, at a temperature of 36 °C. For AP recordings, the concentration of CaCl2 in the bath solution was increased to 1.8 mM. During potassium current recordings, L-type calcium current was blocked by the addition of 2 µm nifedipine to the superfuse. Borosilicate glass micropipettes (1.5–3 MΩ) were filled with pipette solution containing (in mM): 100 K+-aspartate, 40 KCl, 5 MgCl2, 5 EGTA, 5 HEPES, pH adjusted to 7.2 with KOH. APs were recorded with perforated whole-cell patch techniques. APDs were measured from the average of the last 10 (steady-state) APs, obtained during a train of 25 APs at each stimulation rate.

For voltage-clamp experiments, only recordings with an access resistance <20 MΩ were included in the analyses; for measurements of drug-sensitive currents, only cells with less than a 20% change in access resistance were included in the analyses. Series resistance compensation (40–70%) was used for current recordings. All drug-sensitive currents were recorded after 3–5 min of drug superfusion, which in our pilot experiments resulted in steady-state current blockade.

Transient outward potassium current (Ito) was elicited by a series of 100 ms test potentials from −50 to +50 mV, from a holding potential of −60 mV as described previously.17 The steady-state current was used to define the sustained outward K⁺ current. Rapid (Ito) and slow (Ikr) components of the delayed rectifier current were separated using 4-aminopyridine (50 µM) to add to prevent potential contamination with Ikr. Ito was elicited as described previously,16,17 and measured as the 2 mV barium-sensitive current. Ito was defined as the 4-aminopyridine-sensitive sustained outward potassium current and elicited from a holding potential of −40 mV using 10 mV voltage steps from −20 to +50 mV. A −40 mV holding potential with an 80 ms pre-pulse to +30 mV was used to inactivate Ito.13 The sustained 4-aminopyridine-sensitive Ito was measured as the steady-state difference current, recorded after a minimum of 4 min of superfusion with 4-aminopyridine.

2.3.1 L-type calcium current

Conventional whole-cell patch-clamp techniques were used for the measurement of calcium currents (Ica,L). Ica,L was measured using standard voltage step protocols, as described previously.18 Due to the significant alterations in the AP waveform, we observed during HF, AP clamp with both control and HF AP waveforms was used to elicit Ica,L. APs with the median APD90 were selected as the stimulation waveform. AP clamp with a train of 10 APs was used to elicit steady-state Ica,L. The pipette solution contained (mM): 125 CsCl, 20 tetraethylammonium chloride, 5 MgATP, 3.6 creatine phosphate, 10 HEPES, and 50 µM EGTA, pH 7.2. The bath solution (35 °C) contained (mM): 157 tetraethylammonium chloride, 1 CaCl2, 0.5 MgCl2, 10 HEPES, pH 7.4. Ica,L recordings began 3 min after patch rupture. Preliminary experiments with nifedipine confirmed that
was isolated by these methods, and the current was quantified by integrating the area under the elicited current curve (ClampFit v.10, Axon Instruments).

2.4 Western blots

K⁺ channel subunits were assessed by immunoblot analysis. Twenty micrograms of protein from tissue homogenates were subjected to 4–20% SDS–PAGE, blotted onto nitrocellulose membranes (Bio-Rad Labs, Hercules, CA, USA). Anti-Kv1.5, -Kv channel interacting protein 2 (KChIP2), and -Kir2.1 antibodies were from Santa Cruz Biotech (Santa Cruz, CA, USA); anti-Kv4.3, -Kir2.3 and -angiotensin-1 (AT-1) receptor antibodies were from Alomone Laboratories (Jerusalem, Israel). Each sample was normalized to a protein control (GAPDH; Abcam, Cambridge, MA, USA) to normalize loading of each sample. Blots were developed with Super Signal West Pico (Pierce) and quantified using ImageJ (NIH, USA) and Origin 7 (OriginLab, Northampton, MA, USA) software.

Figure 1  Four months of continuous RV tachypacing results in irreversible systolic dysfunction and a substrate for inducible AF. (A) Fractional shortening (%) data obtained from nine dogs, monitored during the tachypacing period (HF, 16 weeks) and during recovery after cessation of tachypacing for up to 7 weeks. (B) Representative ECG and atrial electrogram recorded simultaneously during persistent AF induced by programmed electrical stimulation.
2.5 Measurement of fibrosis

Atrial sections from canine heart were prepared as described previously and stained with Masson’s trichrome to quantify fibrosis. The tissue sections were visualized using a Nikon microscope (TE2000-U, Japan) and areas occupied by blue pixels (fibrosis) were quantified using MetaMorph image analysis software (Molecular Devices, CA, USA).

2.6 Solutions and chemicals

All chemicals for buffer and stock solution preparation were purchased from Fisher Scientific (USA), Sigma Aldrich (St Louis, MO, USA), and Invitrogen Inc. (Carlsbad, CA, USA). Stock solutions of nifedipine, amphotericin B, and 4-aminopyridine were prepared fresh daily. Isoproterenol solutions were prepared daily from commercially available injectable solutions (Sanofi Winthrop Pharmaceuticals, New York, NY, USA). D-Sotalol was obtained from Merck Research Laboratories (West Point, PA, USA). All nifedipine, isoproterenol, and amphotericin B solutions were protected from exposure to light.

2.7 Statistical analysis

Acquired data were analysed using Clampfit 8.0 (Axon Instruments) and Origin 6.1 (OriginLab). Currents were normalized to cell capacitance and are expressed as pA/pF. Action potential durations (APDs) and current densities were analysed by ANOVA with post hoc least significant difference testing as appropriate (SAS for Windows v9.1, Cary, NC, USA). All data are presented as mean ± SE.

3. Results

3.1 Pilot studies

In pilot studies, we observed irreversibility of the fractional shortening induced by 16 weeks of RV tachypacing (Figure 1). Compared with baseline, RV tachypacing produced significant reductions in fractional shortening, and when tachypacing was stopped after 16 weeks, fractional shortening remained significantly reduced from baseline for 5–7 weeks (P < 0.05). Thus, our model of RV tachypacing-induced HF results in irreversible systolic dysfunction. At the same time point (16 weeks of RV tachypacing), AF was induced by atrial premature stimulation and was sustained for 3 months in all three animals tested (Figure 1).

3.2 Main study

3.2.1 Cardiac structure and function

After 4 months of RV tachypacing, there were significant alterations in ventricular function and dimensions (Table 1, P < 0.05), accompanied by significant reductions in LA emptying (FAC, P < 0.05). Haemodynamic alterations were consistent with HF (Table 1).

There was a significant HF-induced increase in LA interstitial fibrosis from 0.54 ± 0.03% in controls to 9.3 ± 0.28% in HF (P < 0.05). The distribution of the fibrosis in HF samples was diffuse, with focal regions of endocardial fibrosis (see Supplementary material online, Figure S1). Myocytes from the LA appendage of the HF group were hypertrophied; capacitance increased from 95 ± 4 pF in controls to 146 ± 11 pF in HF (P < 0.05).

3.2.2 Atrial refractoriness and atrial myocyte APs

In vivo atrial ERPs were significantly shorter in the HF group compared with controls (Figure 2A, P < 0.05), which was paralleled by a reduction in atrial myocyte APDs (Figure 2B). Depolarization of the resting potential was seen in the HF atria group compared with controls (HF atria: −68 ± 3 mV vs. control: −74 ± 2 mV, P < 0.05). The APDs at 50 and 90% repolarization (APD50 and APD90, respectively) were significantly shorter in the HF atrial myocytes compared with controls (Figure 2C and D).

3.2.3 Potassium currents and channels

I_{Kr} was increased in myocytes from the HF atria group (Figure 3, P < 0.05 vs. control). HF significantly reduced I_{Ks} in atrial myocytes relative to controls (Figure 4A and B, P < 0.05). The inward component of the inward rectifier current (I_{K1}) was reduced in HF (Figure 4C and D), whereas outward I_{K1} at −60 mV did not differ between the two groups (0.4 ± 0.02 vs. 0.39 ± 0.03 pA/pF in control and HF, respectively). Measurements of I_{K1} and I_{Ks} revealed a significant HF-induced reduction in I_{Ks}, with no effect on I_{K1} (Figure 5). Alterations in ion currents were further assessed by measurement of ion channel subunits. HF induced no change in Kv4.3, but significantly increased KChIP2 expression (Figure 6). Both Kv1.5 and Kir2.1 were significantly reduced during HF, whereas Kir2.3 was preserved.

3.2.4 Calcium current

Since the APD during HF was significantly attenuated, we hypothesized that this would alter sarcolemmal I_{Ca}. Therefore, L-type calcium current was recorded using representative (median) AP waveforms (shown in Figure 2B) to assess whether HF-induced attenuations of the APD reduced sarcolemmal calcium current. Application of each AP waveform to was increased in myocytes from the HF atria group (Figure 7A). In control myocytes, there was no significant change in the peak amplitude of I_{Ca,L} with the two AP waveforms. However, application of the HF AP to the HF myocytes significantly attenuated peak I_{Ca,L} relative to the control AP in HF myocytes (Figure 7B, P < 0.05). Measuring the integral of the area under the

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*P < 0.05.
peak \( I_{\text{Ca-L}} \) trace (Figure 7C) showed that the HF AP waveform significantly reduced the overall sarcolemmal calcium flux in both control and HF myocytes, but to a significantly greater extent in the HF myocytes (Figure 7, \( P < 0.05 \)). There was no difference in \( I_{\text{Ca-L}} \) measured with standard voltage step protocols (Figure 7D).

### 3.2.5 Redox modulation

Oxidative stress has been implicated in some forms of atrial electrophysiological remodelling.\(^{10,20}\) Therefore, we treated aliquots of myocytes with NAC to assess whether electrophysiology was altered by improving redox balance. Treatment with NAC did not alter \( \text{APD}_{50} \), \( \text{APD}_{90} \), \( I_{\text{to}} \), or the sustained outward \( K^+ \) current in control myocytes (\( n = 6 \)). In HF myocytes, incubation with NAC restored \( \text{APD}_{50} \),...
whereas APD90 was unchanged (Figure 8). To further investigate the potential current alterations by which NAC incubation partially restored the APD in HF myocytes, $I_{K1}$, $I_{to}$, and $I_{Kur}$ were measured after incubation of HF myocytes in NAC (Figure 8D). The HF-induced increase in $I_{to}$ density was restored back towards control values by NAC incubation ($P \nottag NS$ vs. control). NAC did not have any effect on $I_{K1}$ or $I_{Kur}$ density. Angiotensin signalling has been implicated in atrial oxidative stress, and we observed an increase in AT-1 receptor expression during HF.

4. Discussion

HF and AF are increasingly common diseases, with the prevalence projected to continue increasing in the coming decades. Both diseases result in significant morbidity and mortality. Importantly, the addition of one of these diseases to the other increases the risk of death. Potential mechanisms through which HF may promote AF include increased atrial interstitial fibrosis and/or electrophysiological alterations. A key finding of our study is that as previously reported for AF-induced AF, chronic HF induces shortening of the atrial AP (and ERP) and results in a substrate for persistent AF.

We report that compared with short-term HF, chronic HF results in a distinct form of atrial electrophysiological remodelling and provides a substrate for inducible sustained AF. Notably, the underlying ion current alterations differ when comparing chronic AF and short-term HF. Previous studies of HF-induced atrial remodelling have utilized short-term canine ventricular tachypacing. Of note, Nattel and co-workers evaluated canine atrial structural and electrophysiological changes following 5 weeks of ventricular tachypacing to induce HF, and on average, inducible AF was minutes in duration. The primary electrophysiological changes in this model were slowed electrical conduction, atrial APD prolongation accompanied by a modest decrement in $I_{Ca}$, and significant reductions in $I_{to}$ and $I_{Ks}$. There is also evidence in this canine HF model of increased atrial interstitial fibrosis, which may also contribute to the substrate for AF. Our model resulted in a substrate for persistent AF; spontaneous AF was not observed, which is consistent with the previous reports in shorter-term tachypaced canine HF.

We hypothesized that chronic, largely irreversible HF would provide a substrate for persistent AF; this was evident in our pilot studies. This suggests that fundamental changes in the myocardium occurring during chronic HF are associated with the development of an atrial substrate for persistent arrhythmias. The major differences between our results and those of the preceding studies in short-term HF are: the ability to induce sustained persistent AF in some animals and the lack of reversion to normal LV systolic dysfunction after termination of RV tachypacing. All animals...
completed the 4-month period of RV tachypacing, in contrast to the higher rates of death or technical failure reported with faster stimulation rates for shorter durations.28 The extent of atrial fibrosis was similar when comparing our results to the earlier reports,6 suggesting that increased atrial fibrosis occurs early and remains a stable feature during HF.

In our model of chronic HF, we observed increases in atrial $I_{Ca}$ and reciprocal shortening of the APD, accompanied by decreases in $I_{K1}$, $I_{Kur}$, and $I_{Ks}$. Furthermore, the HF-induced decrements in APD resulted in reduced calcium flux. The differences in the observed direction of change in the atrial APD (and the underlying differences in ion currents), compared with that reported earlier,7 suggest that there may be HF duration-dependent atrial remodelling. Further experimentation is required to systematically address this possibility.

Intriguingly, the shortening atrial refractoriness which we observed is consistent with the ‘first factor’ of atrial remodelling thought to promote the perpetuation of AF.29 The seminal studies which provided the evidence for the ‘first factor’ are models of sustained AF where continuous high-rate activity (lone AF models) significantly reduced the duration of the atrial ERP24,30,31 providing a positive feedback loop, whereby transient AF becomes persistent.24 However, although the direction of change in atrial repolarization and refractoriness we observed is similar to reports utilizing either lone AF models32 or myocytes from humans.
with chronic AF, the underlying mechanisms are not entirely consistent. For example, \( I_{\text{to}} \) is reduced in studies of AF-induced atrial remodelling, \( I_{\text{kur}} \) (\( I_{\text{kur}} \)) is variably altered, and \( I_{\text{Ca-L}} \) is also reduced during AF-induced atrial remodelling. In contrast, our findings of a large increase in \( I_{\text{to}} \) with an associated reduction in APD suggest that in chronic HF, electrophysiological mechanisms distinct from AF-induced atrial remodelling result in the same AP phenotype: attenuation of atrial refractoriness. Unlike the reductions in \( I_{\text{Ca}} \) described in AF-induced atrial remodelling, our data suggest that in chronic HF, the intrinsic properties of sarcolemmal \( I_{\text{Ca}} \) are maintained (Figure 7). However, AP clamp experiments indicate a reduction in \( I_{\text{Ca}} \) elicited during an HF AP. We suggest that the acceleration of early repolarization (e.g. an HF-induced reduction in APD from \( \approx 27 \) to \( 11 \) ms) limits sarcolemmal \( I_{\text{Ca}} \) flux, which could itself contribute to further shortening of the AP. A recent paper describing right atrial myocyte electrophysiology in cells from humans with LV systolic dysfunction reports a decrease in refractoriness and APD, with associated reductions in \( I_{\text{to}} \) and preserved \( I_{\text{Ca-L}} \) (measured by voltage step protocol) and \( I_{\text{K1}} \). Collectively, this suggests the possibility of an AP phenotype (i.e. shorter atrial ERP and APD) which may be common to the disease-induced (i.e. AF or chronic HF) atrial substrate for the perpetuation of persistent AF.

The reductions in APD and the atrial ERP were substantial and could potentially shorten the ‘wavelength of re-entry’ thereby increasing the number of arrhythmia wavelets during AF, as has been postulated to occur in AF-induced atrial remodelling. Thus, the persistence of AF which we observed, relative to other shorter duration HF studies, may have resulted from the attenuated refractoriness of the atria during HF. The shortened APD and atrial ERP in HF may have modulated not only arrhythmia persistence, but also atrial contractility. The AP shortening and shortening of the atrial ERP are consistent with the observed attenuation of atrial contractility. APD attenuation also resulted in reduced sarcolemmal calcium flux during HF; this was directly assessed using AP clamp, and in vivo we observed an associated reduction in atrial contractile function. In addition to the reduced \( I_{\text{Ca}} \) which we observed, it has also been reported that there are abnormalities in calcium handling which also contribute to atrial contractile dysfunction during HF, and thus multiple factors may contribute to atrial contractile dysfunction during HF. Hypothetically, this suggests that any intervention attenuating atrial electrophysiological remodelling has the potential to reduce atrial arrhythmia persistence as well as improving atrial contractility.

Our western blot analyses provide some insights into the HF-induced alterations in ion currents. We observed increased \( I_{\text{to}} \), and examination of the channel subunits indicates that \( I_{\text{kur}} \) (\( I_{\text{kur}} \)) was unchanged, whereas the expression of the regulatory \( \beta \)-subunit KChIP2 was significantly increased. KChIP2 is known to increase Kv4-mediated ion currents, and contributes to regional differences in \( I_{\text{to}} \) in canine myocardium. Thus, our data suggest that increased KChIP2 contributes, in part, to the increased atrial \( I_{\text{to}} \) observed during chronic HF. However, the restoration of \( I_{\text{to}} \) with NAC incubation also suggests a role for redox modulation of \( I_{\text{to}} \) amplitude during chronic HF.

We observed a reduction in \( I_{\text{kur}} \) and the putative channel subunit, \( \text{Kv1.5} \), was also reduced. Interestingly, KChIP2 has...
been shown to modulate Kv1.5 trafficking.48 Reduced channel expression, alone or in combination with reduced trafficking to the membrane (as would be predicted from the increased KChIP2 expression), may have resulted in reduced atrial \( I_{\text{Kr}} \) during HF. The reduction in inward \( I_{\text{K1}} \) observed in our data indicates altered current rectification. The reduced expression of the pore-forming channel subunit, Kir2.1, with preserved Kir2.3 expression may have contributed to the observed reduction in \( I_{\text{K1}} \) rectification.49 Thus, many of the observed alterations in ion channels are consistent with HF-induced alterations in ion channel subunit expression.

Oxidative stress has been implicated in some forms of atrial electrophysiological remodelling.10,20 Atrial remodelling due to high-rate activity of the atrium results in mitochondrial dysregulation and oxidative stress.10,21,50,51 Angiotensin-II is increased in HF and has been suggested to contribute to adverse remodelling of the atria through pro-fibrotic, pro-oxidant, and possibly electrophysiological effects,21,50,52,53 (reviewed by Ehrlich et al.44); notably, we observed an HF-mediated increase in LA AT-1 receptor expression.

A recent report indicates that glutathione is reduced in LA samples from patients with chronic HF, suggesting a role for abnormal redox balance in human HF.55 We used NAC, a glutathione precursor,56 to examine whether there was redox modulation of atrial electrophysiology during chronic HF. We observed a partial normalization of atrial electrophysiology with NAC treatment remain undefined at the present time and further experimentation will be required to address the underlying mechanisms.

4.1 Limitations

Our model of HF is a non-ischaemic model of cardiomyopathy, and HF patients have multiple aetiologies of disease. Our animals were not treated with beta-blockers, ACE-inhibitors, or diuretics, as would occur in human HF. We studied a single duration of HF which is unlikely to reflect time-variant atrial remodelling. We only studied myocytes from the LA appendage and cannot rule out the contributions of other regions of the atria or pulmonary vein sleeves to the aetiology of AF during chronic HF.

Focal mechanisms appear to contribute to the initiation of AF during canine tachypacing-induced HF.3,43,57 Our studies addressed the substrate for persistence of AF and did not address mechanisms of initiation of AF. Although we observed reduced in vivo atrial contractility, we did not evaluate specific abnormalities in calcium handling or basic contractile properties, as contributors to impaired contractile function.

5. Summary and conclusions

Chronic RV tachypacing results in irreversible HF and atrial current remodelling that is distinct from that reported in short-term HF studies. Chronic HF results in shortening of atrial ERP, and the APD, which is attributable in part to increased \( I_{\text{to}} \). AP remodelling in HF reduces sarcosomal \( I_{\text{CaL}} \) flux and was associated with impaired atrial contractility. We suggest that the duration of HF is a critical modulator of atrial remodelling processes and ultimately of the persistence of AF.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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