Anatomy & Physiology: Current Research
ISSN: 2161-0940

Dobrzynski et al., Anat Physiol 2015, 5:1
DOI: 10.4172/2161-0940.1000164

Research Article Open Access

Molecular Investigation into the Human Atrioventricular Node in Heart Failure

Halina Dobrzynski[1*], Andrew Atkinson[1], Zoltan Borbas[1], Christina M Ambrosi[2] and Igor R Efimov[2*]

[1]*Institute of Cardiovascular Sciences, University of Manchester, 46 Grafton Street, Manchester, UK*

[2]*Department of Biomedical Engineering, Washington University, One Brookings Drive, St. Louis, MO, USA*

[*]**Corresponding authors:** Halina Dobrzynski, Institute of Cardiovascular Sciences, University of Manchester, 46 Grafton Street, Manchester, M13 9NT, UK, Tel: +44 (0)161-275 1182; E-mail: halina.dobrzynski@mancheser.ac.uk

Igor R. Efimov, Department of Biomedical Engineering, Washington University, Campus box 1097, One Brookings Drive, St. Louis, MO 63130, USA, Tel: 5-8612; E-mail: igor@wustl.edu

Rec Date: Jul 23, 2014; **Acc Date:** Nov 17, 2014; **Pub Date:** Nov 20, 2014

Abstract

The atrioventricular node (AVN), the molecular basis has been studied in animal models; however, the human AVN remains poorly explored at the molecular level in heart failure patients. We studied ex vivo donor human hearts rejected for transplantation (n=6) and end-stage failing hearts with cardiomyopathy of various etiologies (n=6). Microdissection and quantitative PCR (qPCR) were used to anatomically map mRNA expression in both failing and non-failing hearts from tissue sections through the AVN, atrial and ventricular muscle. In the failing ventricle significant (P<0.05) downregulation is apparent for vimentin, hERG and Kir3.4 and trend to downregulation for Nav1.5, Cav1.2, Tbx3, Kir2.1, NCX1, Cx43 and Cx45. In the failing atrium, there is non-significant trend in upregulation for Nav1.5, HCN2, Cav3.1, Kv1.5, Kir3.1, RYR2 and significant upregulation for Cx40 and trend in downregulation for hERG, Kir2.1 and NCX1, and significant downregulation for HCN4 and Kir3.4. In the failing AVN there is significant (P<0.05) downregulation for vimentin, collagen, Tbx3, Kir3.1, Kir3.4, Cx45 and HCN4; there is also trend toward downregulation for HCN2, Kv1.5, Kir2.1 and RYR2. In the failing AVN there is significant (P<0.05) upregulation for Cx40, HCN1 and Cav3.1; and trend in upregulation for Nav1.5 and Cx43. For several transcripts, we also analysed corresponding protein expression via immunofluorescence. The protein expression data on the AVN for HCN1, Cav3.1 and Cx40 support qPCR data. Remodelling of AVN in heart failure might contribute to prolonged AV conduction time and could explain the prolonged PR interval that occurs in heart failure patients.

Keywords: AVN; Cardiomyopathy; Molecular biology; Heart failure

Introduction

Heart failure (HF) is a severe pathological condition, associated with high morbidity and mortality. HF is the condition in which the heart is unable to provide cardiac output sufficient for the body's metabolic requirement. HF is often the final outcome of several years of worsening cardiomyopathy, and spectra of etiologies underlying HF cover a wide range of common conditions, for example coronary heart disease (CHD), which affects approximately 2.5 million people in the UK and 5.7 million of people in the USA [1,2]. Treatment may have little effect on the final outcome of HF [3], as the etiology of the disease and its underlying mechanisms are complex and not fully understood.

Electrocardiography (ECG) and echocardiography are common tools, which aid diagnosis of HF. Bradycardia is not usually observed clinically in HF as it is masked by increased β-adrenergic drive. The PR interval is often prolonged in HF indicating slowed conduction through the atrioventricular node (AVN) [4]. First or second-degree AV block in HF patients increases a risk factor for sudden cardiac death [4,5].

The AVN is an important component of the cardiac conduction system and is responsible for the conduction of the action potential from the atria to ventricles, and for introducing a delay between atrial and ventricular systole required for complete ventricular filling for subsequent efficient pumping of blood. In the human heart, the AVN is delineated by the triangle of Koch in the floor of the right atrium, bordered by the coronary sinus; the tendon of Todaro and the tricuspid valve (Figure 1) (compared with Figure S1). Tawara in 1906 first described the complex structure of the AVN as a histologically discrete group of cells at the proximal end of the bundle of His which he called the "Knoten" [6].

The AVN is a complex region of the heart and it contributes to the development of arrhythmias and cardiac dysfunction. The molecular and structural basis for remodelling of the AVN in HF is poorly explored, especially in the human heart. It is generally believed that the compact node [6] is responsible for most of the conduction delay between excitation of the atria and ventricles, and thus we investigated this region at the structural and molecular level in donor and end-stage failing human hearts. We hypothesizes that ECG changes (changes in the PR interval) observed in HF patients are based on structural remodelling, as well as remodelling of proteins and genes underlying ion channels, Ca^{2+}-handling proteins, and gap junctions in the failing AVN. This study is the first comparative study of the AVN in failing and non-failing human heart.

Methods

The use of human hearts for research was approved by the Institutional Review Board of Washington University in Saint Louis. Failing hearts (n=6) with cardiomyopathy were obtained during transplantation at the Barnes-Jewish Hospital, Washington University School of Medicine (Saint Louis, MO). We also used non-failing donor hearts (n=6), which were rejected for transplantation for various

reasons including age, early stage hypertrophy, atrial fibrillation, and coronary disease. Donor hearts were provided by the Mid-America Transplant Services (Saint Louis, MO).

Histology

We obtained cryosections from the compact AVN and adjacent atrial muscle (AM) and ventricular muscle (VM) from six donor and six failing human hearts. The profile of patients from whom tissue samples were obtained for histology, qPCR and immunohistochemistry analysis is summarized in Table 1. We used histology to determine the location of the AVN. Masson's Trichrome staining was carried out as previously described [7,8].

Patient	Age	Gender	Disease Category	Cause of death
1	58	F	Donor	Intracranial hemorrhage
2	70	M	Donor	Intracranial hemorrhage
3	40	M	Donor	Brain tumor
4	72	F	Donor	Intracerebral hemorrhage
5	33	F	Donor	Car accident
6	55	F	Donor	Stroke
7	43	M	Failing	Idiopathic dilated cardiomyopathy
8	37	F	Failing	Idiopathic cardiomyopathy
9	62	M	Failing	Ischaemic cardiomyopathy
10	52	F	Failing	Non-ischaemic cardiomyopathy
11	45	F	Failing	Hypertrophic obstructive cardiomyopathy
12	50	M	Failing	Ischaemic cardiomyopathy

Table 1: Profile of patients from whom tissue samples were obtained.

Quantitative PCR

For qPCR, we micro-dissected the AVN (CN), AM and VM (as outlined in Figure 1), from ~10 frozen sections (16 µm thick) (adjacent to those shown in Figure 1), which underwent a rapid haematoxylin and eosin staining (H&E) protocol [8]. We did not study other components of the AV conduction axis (e.g., INEs and His bundle) as there was not enough human material available. Following extraction of total RNA from the samples and reverse transcription to produce cDNA, the abundance of cDNAs for a selected cell type-marker, e.g., Tbx3, ion channels, gap junction channels and Ca^{2+}-handling proteins were measured using qPCR. The data were analyzed using the '?CT technique': to correct for variations in the amount of total RNA in the different samples, the amounts of the different cDNAs measured were normalized to the abundance of a housekeeper gene (ribosomal protein 28S, which proved to be more reliable than GAPDH as shown in our earlier studies [7,8]. Means ± SEM are shown. Significant differences in the abundance of mRNAs in different tissues were identified using ANOVA. A more detailed description of the methods can be found in the supplemental data (SD).

Immunohistochemistry

Immunohistochemistry was performed as previously described on the human sinus node and AVN respectively [7,8] using antibodies listed in Table S4.

Results

Histology of human atrioventricular node

We stained tissue sections obtained from 12 hearts. Figure 1 show Masson's trichrome stained tissue sections from one donor heart (left panel) and one failing heart (right panel), respectively. Figure 1 shows that in HF the AVN becomes hypoplastic compared to the donor AVN. Adjacent tissue sections were stained with H&E and were used for microdissection (Figure S1) for qPCR experiments as mentioned above and described in more details in the SD.

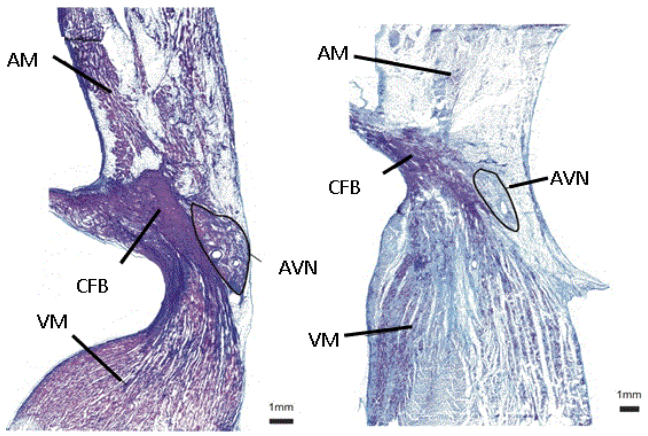

Figure 1: Histology of human atrioventricular node. Masson's trichrome staning through donor AVN (left panel) and failing AVN (right panel). AVN: Atrioventricular Node; AM: Atrial Muscle; CFB: Central Fibrous Body; VM: Ventricular Muscle.

qPCR data

Table S1 shows all targets investigated in the compact AVN, AM and VM. We have investigated other transcripts not listed in Table S1 [8], but we were unable to amplify these transcripts due to limited amount of human tissue.

Tbx3, vimentin and collagen

Tbx3, a transcription factor expressed in the cardiac conduction system [8-10], was used to characterize the tissues studied by qPCR. Tbx3 is highly expressed in the AVN [7]. Figure 2 shows that the expression of Tbx3 is significantly higher in the AVN of the donor heart than in the working myocardium. Significant downregulation of this mRNA is observed in the failing AVN compared to the donor AVN.

Vimentin (marker of fibroblasts) and collagen mRNAs are significantly downregulated in the failing AVN compared to donor AVN (Figure 2) (compared with Figure S2), and these data therefore suggest that there is remodelling of extracellular matrix in the failing AVN.

Nav1.5 (cardiac Na^+ channel)

The cardiac Na^+ channel, Nav1.5, is primarily responsible for INa (responsible for the rapid action potential upstroke in the working myocardium) [9]. Figure 2 confirms the expected pattern of expression for Nav1.5 mRNA in the donor cardiac tissues; its expression being lower in the donor AVN compared to donor AM or VM. In the failing VM and AM there was trend towards downregulation compared to the donor tissues, whereas in the failing AVN there was trend towards upregulation of this mRNA (Figure 2).

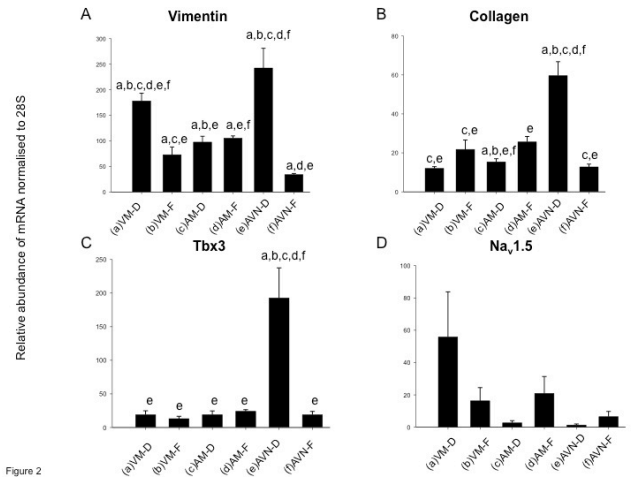

Figure 2: qPCR results for Vimentin, Collagen, Tbx3 and Nav1.5 mRNA and significance data. Means+SEM (n=6 from donor hearts and n=6 form failing hearts) shown. Significant differences ($P<0.05$) amongst different tissue are represented by letters above bars. a, VM-D; b, VM-F; c, AM-D; d, AM-F; e, AVN-D; f, AVN-F. AM: Atrial Muscle; AVN: Atrio Ventricular Node; D: Donor; F: Failing; VM: Ventricular Muscle.

HCN1, HCN2 and HCN4 (pacemaker channels)

HCNs genes encode for the pacemaker current, If. In small mammals If is present in the cardiac pacemaker tissues and generally not in the working myocardium [11]. Figure 3 confirms the expected distribution of HCNs mRNAs in donor cardiac tissues, with significantly higher expression in the donor AVN compared to the donor AM and/or VM. Significant upregulation of HCN1 and HCN4 are found in the failing AVN compared to the donor AVN, whereas a trend in downregulation of HCN2 found in the failing AVN compared to the donor AVN (Figure 3).

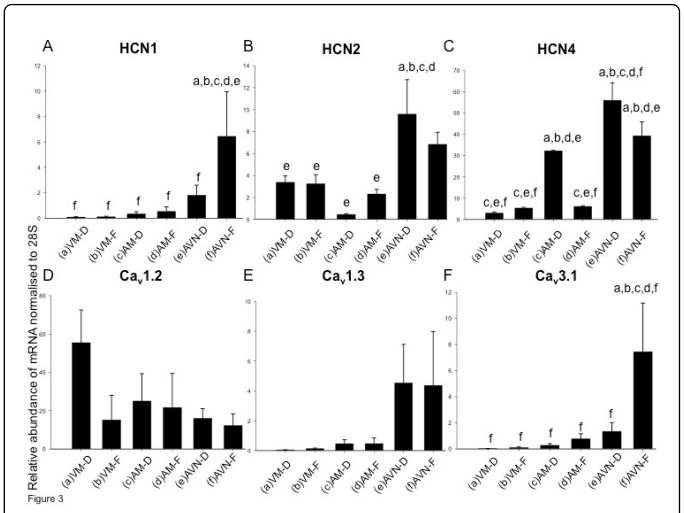

Figure 3: qPCR results for HCN1, HCN2, HCN4, Cav1.2, Cav1.3 and Cav3.1 mRNA and significance data. See Figure 2 for details.

Cav1.2, Cav1.3 and Cav3.1 (Ca^{2+} channels)

Two separate Ca^{2+} currents have been recorded from cardiac myocytes, the L-type (ICa,L) and T-type (ICa,T) [12]. Cav1.2 and Cav1.3 are responsible for ICa,L and Cav3.1- Cav3.3 are responsible for ICa,T [9]. Figure 3 confirm expected distribution of Cav1.2 and Cav1.3 mRNAs across the three regions investigated: Cav1.2 mRNA expression is greater in the donor VM than in the donor AVN, whereas Cav1.3 mRNA expression is greater in the donor AVN than in the donor VM. Downregulation of Cav1.2 mRNA expression is observed in the failing VM compared to the donor VM (Figure 3). Figure 3 also confirms expected distribution of Cav3.1 mRNA in donor cardiac tissues: Cav3.1 mRNA expression is lower in the donor VM and AM compared to the donor AVN. In the failing AVN there is significant upregulation compared to the donor AVN (Figure 4). In addition, this figure illustrates that at the protein level there is higher expression of HCN1 and Cav3.1 in the failing AVN compared to the donor AVN.

Kv1.5, ERG, Kir2.1, Kir3.1 and Kir3.4 (K^+ channels)

There are three delayed rectifier K^+ currents, the ultra-rapid (IK,ur), rapid (IK,r) and slow (IK,s). The ion channels responsible for IK,ur, IK,r and IK,s are Kv1.5, hERG and KvLQT1, respectively. IK,ur is considered a major repolarizing current in the human atrium [7]. Figure 5 confirms the expected distribution of Kv1.5 mRNA in donor cardiac tissues: greater expression is evident in the donor AM and AVN compared to the donor VM. A trend of downregulation of Kv1.5 mRNA is observed in the failing AVN compared to the donor AVN, whereas a trend to upregulation is seen in the failing AM and VM compared with controls (donor tissues). IK,r and IK,s, are responsible for repolarization in the working myocardium in various species including the human [7]. Figure 5 confirms the expected distribution of hERG mRNA in donor cardiac tissues: higher expression in the donor VM compared to the donor AM and AVN. Significant downregulation occurs in the failing VM compared to the donor VM (Figure 5). Unfortunately, KvLQT1 mRNA failed to amplify in some cardiac tissues as well as other K^+ channels such as Kv1.4, Kv4.2 and Kv4.3 responsible for outward K^+ current, IK, too (Table S1).

Kir2.1 is responsible for t0068e background inward rectifier K^+ current (IK,1) [9]. Figure 5 confirms the expected distribution of Kir2.1 mRNA in donor cardiac tissues: significantly greater expression is evident in the donor VM compared to the donor AVN. A trend toward downregulation of this mRNA is observed in the failing cardiac tissues compared to their donor counterparts (Figure 5).

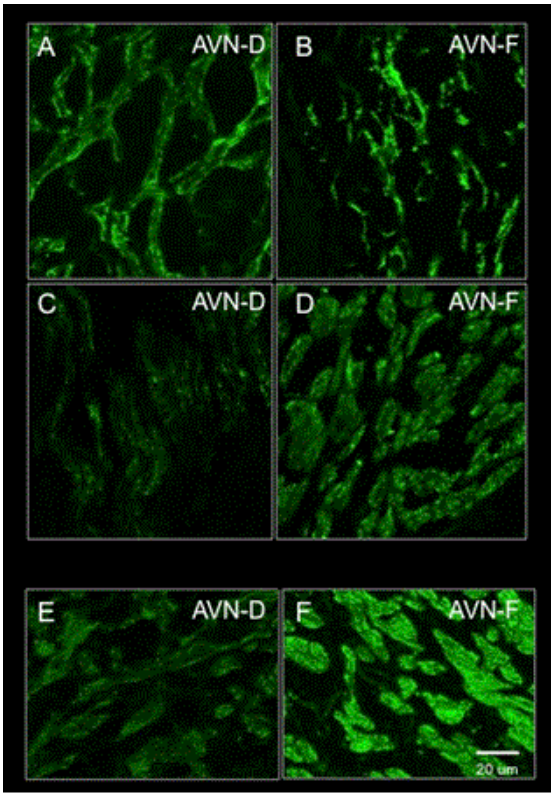

Figure 4: Immunolabelling results for HCN1 and Cav3.1 in the atrioventricular node. Top panels show expression of HCN1 in the donor AVN (left panel) and stronger expression in the failing AVN (right panel). Bottoms panels show expression of Cav3.1 in the donor AVN (left panel) and stronger expression in the failing AVN (right panel). The images are from patients 6 and 10, see Table 1 for details.

The ACh-activated K^+ current, IKACh, is generated by a heteromultimer of Kir3.1 and Kir3.4 [13]. Figure 5 confirms the expected distribution of Kir3.1 and Kir3.4 mRNAs in donor cardiac tissues: significantly lower expression in the donor VM compared to the donor AVN (significant for Kir3.4). Kir3.1 mRNA is downregulated in the failing AVN compared to the donor AVN (Figure 5). Kir3.4 mRNA expression is significantly downregulated in the failing VM and AVN tissues (Figure 5).

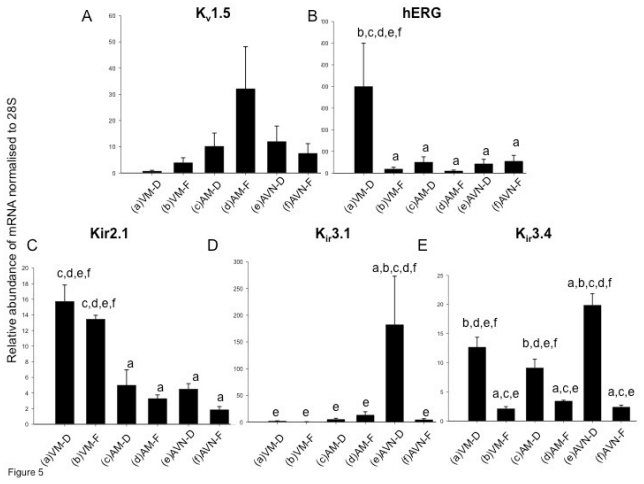

Figure 5: qPCR results for Kv1.5, ERG, Kir2.1, Kir3.1 and Kir3.4 mRNA and significance data. See Figure 2 for details.

Ca^{2+} handling proteins RYR2 and NCX1

Intracellular Ca^{2+} has been suggested to play an important role in pacemaking in small mammals [14]. Figure 6 shows that the distribution of the SR Ca^{2+} release channel RYR2, mRNA is higher in the donor AVN compared to the donor working myocardium; a trend of downregulation of this mRNA is observed in the failing AVN compared to donor and a trend for upregulation in the failing AM. Figure 6 also shows the distribution of the Na^+-Ca^{2+} exchanger, NCX1 mRNA expression is relatively consistent across all regions investigated, but a trend towards higher expression is observed in the donor VM (Figure S3 in supplementary).

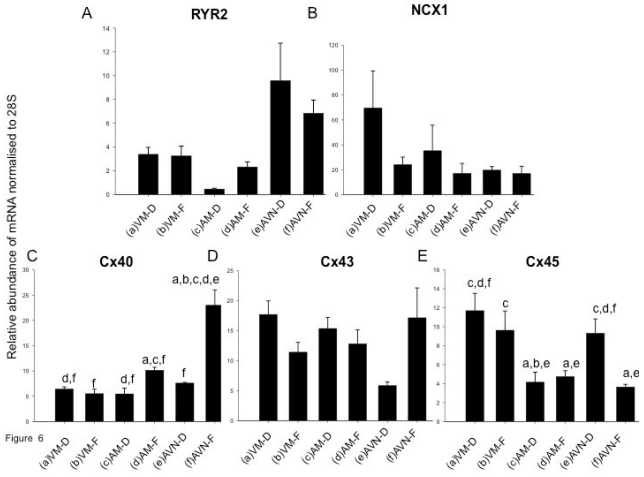

Figure 6: qPCR results for RYR2, NCX1, Cx40, Cx43 and Cx45 mRNA and significance data. See Figure 2 for details.

Figure 7 illustrates that at the protein level there is lower expression of RYR2 in the failing AVN compared to the donor AVN, but no changes in NCX1 protein. In the failing VM there appear to be downregulation of RYR2 and NCX1 protein as documented previously [15].

Connexins Cx40, Cx43 and Cx45

Figure 6 confirms distribution of Cx40, Cx43 and Cx45 mRNAs in donor cardiac tissues [7]. There is significant upregulation of Cx40 and trend towards upregulation for Cx43 mRNAs but significant downregulation of Cx45 mRNA in failing AVN compared to the donor AVN (Figure 6).

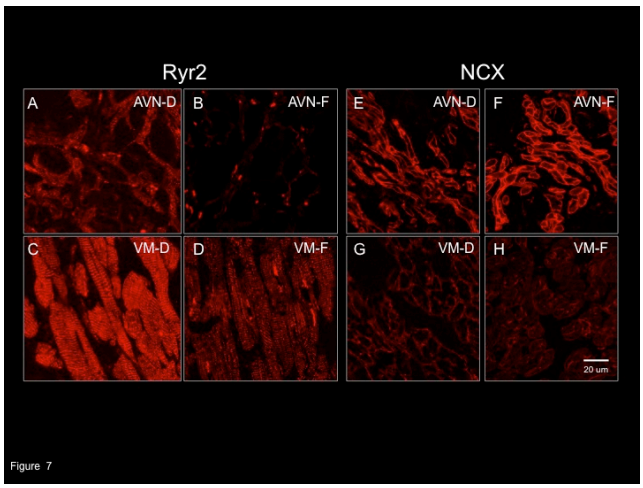

Figure 7: Immunolabelling results for RYR2 and NXC1 in the atrioventricular node and ventricular muscle. A, B, striated pattern of RYR2 expression in the donor AVN (A) and weaker expression in the failing AVN (B). C, D, strong striated pattern of RYR2 expression in T-tubules in the donor VM (C) and weaker expression in the failing VM (D). E, F, NCX1 expression in the membrane of the donor AVN (E) and failing AVN (F). G, H, NCX1 expression in the membrane of the donor VM (G) and failing VM (H). The images are from patients 6 and 10, see Table 1 for details.

Figure 8 further illustrates that there is higher distribution of Cx40 in the failing AVN compared to the donor AVN at the protein level. This figure also illustrates ECG recordings obtained from one healthy and one HF patient, and it shows that failing heart has severe abnormalities in the cardiac conduction system. As can be seen in Figure 8 in HF ECG there is an evidence of first degree AV block (prolonged PR interval). There is also evidence of slow ventricular conduction seen as prolonged QRS interval (Table S2).

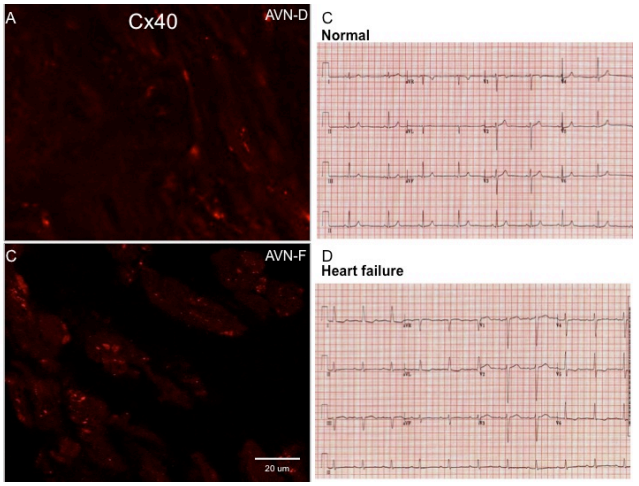

Figure 8: Cx40 immunolabelling in the atrioventricular node and ECG recordings. A, punctuate Cx40 expression in the donor AVN. B, punctuate Cx40 expression in the failing AVN. C. EGC recording from a healthy patient. D. ECG recording from HF patient showing prolongation of PR interval and conduction block in the left bundle branch. AVN-D, atrioventricular node donor; AVN-D; atrioventricular node failing. The images are from patients 6 and 10, see Table 1 for details.

Discussion

The present study is the first to investigate changes in ion channels, Ca^{2+} handling proteins and connexins at mRNA and protein levels in the site of normal regulation of the AV conduction, the AVN, in human failing versus non-failing hearts. The results obtained indicate that changes in expression occur in a number of the genes encoding and proteins underlying key ion channels, Ca^{2+} handling proteins and connexins in this region. Our data also show that there is hypoplasia in failing AVN as well as remodelling of extracellular matrix evident from downregulation of vimentin and collagen. Table S3 shows P values for transcripts investigated and Table S4 summarizes mRNA expression in all regions investigated. The failing ventricle (compared to the donor ventricle) has downregulation for Nav1.5, Cav1.2, hERG (significant), Kir3.1, Kir3.4 (significant), vimentin (significant), Kir2.1, NCX1, Cx43 and Cx45 and upregulation for collagen, HCN4, Cav1.3, Cav3.1 and Kv1.5. In the failing atrium there is upregulation for Nav1.5, HCN2, Cav3.1, Kv1.5, RYR2, Cx40 (significant), collagen (significant) and vimentin and downregulation for HCN4 (significant), hERG, Kir2.1, Kir3.4 (significant), NCX1 and Cx43. In the failing AVN there is significant downregulation for vimentin, collagen, Tbx3, HCN1, HCN4, Kir3.1, Kir3.4 and Cx45; there is also trend toward downregulation for HCN2, Kv1.5, Kir2.1 and RYR2. In the failing AVN there are significant upregulation for Cav3.1 and Cx40; and trend in upregulation for Nav1.5 and Cx43. The protein data on the AVN for HCN1, Cav3.1 and Cx40 support qPCR data. These changes may contribute to the prolonged AV conduction time observed in HF patients and could explain the prolonged PR intervals that can occur in HF patients shown in Figure 8.

Extracellular matrix (ECM) remodelling

The ECM is important in determining myocardial contractility, compliance and conductivity and is altered in many ways in failing hearts. Fibroblasts are the most abundant cells in the heart, synthesizing and regulating ECM components, the foremost of which is collagen. Vimentin is expressed in fibroblasts and proves a useful marker in measuring fibroblast abundance. In HF, trends in collagen abundance differ widely across studies and experimental models. Diffuse fibrosis of the myocardium is typically described in hypertrophic and pressure overload disease models, resulting in impaired systolic and diastolic function [16,17]. Collagen also reduces conductivity, predisposing to tissue to arrhythmias [18]. In our study both vimentin and collagen were downregulated in the failing AVN, suggesting that its conductivity is altered. Downregulation of collaged has been observed in HF sheep heart [19] and the sinus node of old rats [20].

Tbx3, ion channels, connexins, Ca^{2+} handling remodelling

Tbx3 has been identified as being primarily responsible for nodal tissue development. In the early sinus node and AVN, it represses transcription of Cx40 and Cx43 (high-conductance connexins) and Nav1.5 (responsible for the fast inward depolarizing Na^{+} current), as well as other atrial myocyte markers [21,22]. Hoogars et al., demonstrated that Tbx3 deficiency resulted in Cx40, Cx43 and Nav1.5 being expressed in the nodal tissue, and forced expression of Tbx3 in atrial tissue resulted in ectopic functional pacemaker tissue [22]. In our study Tbx3 was significantly downregulated in the failing AVN and perhaps its downregulation can explain the changes we observed in Cx40, Cx43 and Nav1.5 expression, i.e., upregulation in the failing AVN.

In our study we investigated sodium channel Nav1.5, gene name SCN5A. [7] showed that in in the human heart the distribution of Nav1.5 was high in the VM and AM compared to the AVN [7]; this distribution was confirmed by our results from donor samples. Borlak and Thum, reported a decrease in Nav1.5 in the VM from failing human hearts from ischaemic or dilated cardiomyopathy conditions [23]. Our results confirmed decreased Nav1.5 expression in the VM and thus agree with the results of [23]. Nav1.5 expression in the failing AVN was found to be slightly increased, perhaps due to downregulation of its suppressor Tbx3.

Electrical coupling of cardiac cells via connexins is one of the most important factors determining conduction velocity. Cx43 is the most abundant connexin in the heart and produces strong electrical coupling, allowing uniform contraction through rapid impulse transmission. In the nodal tissue there is weak electrical coupling, which allows nodal cells to maintain a less negative resting potential than the surrounding myocardium; without this insulation from the hyperpolarising influence of surrounding working myocardium, pacemaking would be suppressed [24,25]. This weak coupling is achieved through the presence of fewer and smaller gap junctions, absence of Cx43, and higher expression of the low-conductance isoform Cx45 [25]. Cx40 (a high conductance isoform) is expressed in atrial myocardium and in the His-Purkinje system [7]. Connexins play an important role in altered electrophysiology during cardiomyopathy [26]. For example, Cx43 downregulation in working myocardium is associated with HF and arrhythmias [26,27] and such downregulation was observed in our study. Complex alterations in Cx40 expression across the heart have been linked to HF, particularly to bradycardia and atrial fibrillation [28,29]. In our study upregulation of Cx40 and Cx43 and downregulation of Cx45 was seen in the failing AVN, coupled with the downregulation of Tbx3 and collagen, and upregulation of Nav1.5 mentioned above could further reinforce our thinking that there is altered function of the AVN in HF. There results further reinforce the idea that there is altered function of the AVN in HF. Perhaps in the failing heart, the AVN cells adapt a phenotype that is more similar to the working myocardium due to altered level of transcription factors that pattern the conduction system, i.e. Tbx3.

Pacemaker channels

The pacemaker channels investigated were HCN1, HCN2 and HCN4, which contribute to diastolic depolarization of the pacemaker action potential. Work by Greener et al., on the human AVN showed highest expression of HCN4, followed by HCN2 and then HCN1 in the AVN; in the AM and VM there was little expression of HCN1 and HCN4 [7] this distribution was largely confirmed by our results from donor samples. Significant upregulation of HCN1 was observed in the failing AVN, and there was trend toward downregulation of HCN2 and significant downregulation of HCN4. Upregulation of HCN1 could lead to increased pacemaking role of the failing AVN to attempt to stimulate and improved more rhythmical contraction in the ventricles. In the failing atrium there is slight upregulation of HCN1 and HCN2, but significant downregulation of HCN4. In the failing ventricle there was slight upregulation of HCN4, which agrees with study by [23].

Calcium channels

mRNA of both L-type (Cav1.2 and Cav1.3) and T-type calcium channels (Cav3.1) were investigated. Cav1.2 expression in donor tissue agrees with the results of [7,30]: expression appears higher in the AM and VM then in the conduction tissue. The results from Cav1.3 mRNA expression are consistent with Greener et al., findings: low VM expression and high in the AVN [7].

Borlak and Thum, reported a decrease in Cav1.2 in failing ventricle; our findings also show a decrease. Downregulation of L-type Ca^{2+} channels in HF has been well documented [31]. It is known that downregulation of L-type of Ca^{2+} channels contributes to atrial fibrillation by decreasing the ERP and slowing of conduction [31,32].

Cav1.3 has been shown to have a role in pacemaking [33], and Cav1.3-/- mice have been found to display a prolonged PR interval [34]. This explains the abundant expression of Cav1.3 in the donor AVN and correlates well with the role of T-type Ca^{2+} current [35]. In the failing AVN our data show that there is no change in Cav1.3 expression.

Our results showed significant upregulation of Cav3.1 in the failing AVN and this upregulation would, as with HCN1, imply an increased ectopic pacemaking role for the AVN in HF because ICa, T may be important in maintaining speed of diastolic depolarization in pacemaking [36].

Potassium channels

The K+ channels investigated were the delayed rectifier channels Kv1.5, hERG and the inwardly rectifying channels Kir2.1, Kir3.1 and Kir3.4.

Our results agree with the findings of [7,30] concerning of Kv1.5 expression in donor tissue. Kv1.5 is responsible for the ultra-rapid delayed rectifier K^{+} current. In our study, Kv1.5 was upregulated in

failing VM and AM, but downregulated in the failing AVN in contrast to that seen by Borlak and Thum in the failing ventricle.

hERG expression also largely agrees with [7,30]; its expression being highest in the donor VM. hERG is responsible for the rapid delayed rectifier current. Choy et al., reported decreased ventricular hERG expression in HF, which agrees with the results from our study [37]. hERG downregulation may contribute to a prolonged action potential and QT interval which is a hallmark of HF [38]. In addition, Kv1.5 and hERG remodeling in AM and VM (up- and downregulation respectively) could lead to myocardial arrhythmias.

Inwardly-rectifying K^+ current (IK, 1) (formed by Kir2.1-2.3) is present in working myocytes, maintaining resting membrane potential [8]. Kir2.1 has by far the greatest contribution to IK, 1 [39]. In our study we observed downregulation of Kir2.1 in our failing samples compared to donor samples. Downregulation has been observed previously in human HF tissue, which could lead to arrhythmias [23-30].

Our results showed minimal expression of Kir3.1 in the donor VM agreeing with the findings of [30]. Our findings in the donor AVN for Kir3.1 and Kir3.4 were highest of the three regions as previously observed [7]. Both channels showed significant downregulation in the AVN in HF. Expression of Kir3.4 was also significantly downregulated in the diseased working myocardium. Correspondingly, [23] report a decrease in the right ventricle. Kir3.1 and Kir3.4 are inwardly rectifying channels activated by ACh that form IK,ACh. The activation of these channels produces an important rate-modulating effect: as K+ enters via the ACh-activated channels EK decreases, making the cell less likely to depolarize [13]. Therefore downregulation of these channels will make the cells of the AVN in HF more likely to depolarize.

Ca2+-handling proteins

The Ca^{2+}-handling proteins investigated were RYR2 and NCX1. No change was observed in the failing VM. Borlak and Thum, reported a decrease of RYR2 expression in the failing VM while [40] observed non-significant downregulation in the failing VM. RYR2 dysfunction in cardiac failure is well documented [40] hyperphosphorylation of RYR2 appears to be the reason the channels remain open longer [41], releasing more Ca^{2+} which in excess is known to be arrhythmogenic [41]. In the failing AVN, trend towards downregulation was observed. Perhaps this downregulation of RYR2 in the AVN represents an attempt to prevent excess Ca^{2+} release by these channels and thereby prevent arrhythmias.

Our results for NCX1 demonstrate high expression in the donor VM. NCX1 was found to show trend to downregulation in the VM in HF, consistent with the findings of [23]. However, other studies have shown upregulation of both NCX1 mRNA and protein [42,43]. NCX1 is responsible for extrusion of Ca^{2+} out of the cell, in exchange for Na^+. However, when excess [Na^+] occurs, NCX1 can work in reverse mode, extruding Na^+ in exchange for Ca^{2+} [42]. NCX1 is involved in generation of delayed after depolarization leading to arrhythmias, ventricular downregulation of NCX1 may, therefore, be a protective effect against arrhythmogenesis.

Conclusion and Study Limitation

The present study is the first to investigate changes in ion channels, Ca^{2+} handling proteins and connexins at mRNA and protein levels in the AVN, in human failing versus non-failing hearts. The important findings of the present study are: 1) in our study both vimentin and collagen were downregulated, whereas Cx40 and Cx43 expression were upregulated in the failing AVN, suggesting that its conductivity is altered; 2) upregulation of HCN1 and Cav3.1 in the failing AVN imply an increased pacemaking role for the AVN in HF; 3) downregulation of Kir3.1 and Kir3.4 in the AVN in HF suggest that the cells of the failing AVN are more likely to depolarise. These changes may contribute to the changes in the AV conduction observed in HF patients.

We did not study other components of the AV conduction axis (e.g., INEs and His bundle), as there was not enough human material available. For qPCR we were unable to amplify some transcripts (not listed in Table S1) due to limited amount of human tissue available. We were also, for the same reason, not able to carry on more immunohistochemical experiments. Finally, we did not have access to any medical records of these patients and we were no able to perform any functional experiments on the hearts.

Acknowledgments

Project was supported by NIH grants R01 HL085369 and HL114395 (IRE) and the British Heart Foundation (HD).

References

1. Allender S, Peto V, Scarborough P, Kaur A, Rayner M. Chapter 2: Morbidity. Coronary heart disease statistics. 2008; BHF: London.
2. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, et al. (2011) Heart disease and stroke statistics--2011 update: a report from the American Heart Association. Circulation 123: e18-18e209.
3. Cowie MR, Wood DA, Coats AJ, Thompson SG, Suresh V, et al. (2000) Survival of patients with a new diagnosis of heart failure: a population based study. Heart 83: 505-510.
4. Xiao HB, Roy C, Fujimoto S, Gibson DG (1996) Natural history of abnormal conduction and its relation to prognosis in patients with dilated cardiomyopathy. Int J Cardiol 53: 163-170.
5. Schoeller R, Andresen D, Büttner P, Oezcelik K, Vey G, et al. (1993) First- or second-degree atrioventricular block as a risk factor in idiopathic dilated cardiomyopathy. Am J Cardiol 71: 720-726.
6. Tawara S. Das Reizleitungssystem des Säugetierherzens. Eine anatomisch histologische Studie über das Atrioventrikularbündel und die Purkinjeschen Fäden. 1906; Jena: Gustav Fischer.
7. Greener ID, Monfredi O, Inada S, Chandler NJ, Tellez JO, et al. (2011) Molecular architecture of the human specialised atrioventricular conduction axis. J Mol Cell Cardiol 50: 642-651.
8. Chandler NJ, Greener ID, Tellez JO, Inada S, Musa H, et al. (2009) Molecular architecture of the human sinus node: insights into the function of the cardiac pacemaker. Circulation 119: 1562-1575.
9. Greener ID, Tellez JO, Dobrzynski H, Yamamoto M, Graham GM, et al. (2009) Ion channel transcript expression at the rabbit atrioventricular conduction axis. Circ Arrhythm Electrophysiol 2: 305-315.
10. Tellez JO, Dobrzynski H, Greener ID, Graham GM, Laing E, et al. (2006) Differential expression of ion channel transcripts in atrial muscle and sinoatrial node in rabbit. Circ Res 99: 1384-1393.
11. DiFrancesco D (1993) Pacemaker mechanisms in cardiac tissue. Annu Rev Physiol 55: 455-472.
12. Dobrzynski H, Boyett MR, Anderson RH (2007) New insights into pacemaker activity: promoting understanding of sick sinus syndrome. Circulation 115: 1921-1932.
13. Krapivinsky G, Gordon EA, Wickman K, VelimiroviÄ‡ B, Krapivinsky L, et al. (1995) The G-protein-gated atrial K+ channel IKACh is a

heteromultimer of two inwardly rectifying K(+)-channel proteins. Nature 374: 135-141.

14. Bogdanov KY, Vinogradova TM, Lakatta EG (2001) Sinoatrial nodal cell ryanodine receptor and Na(+)-Ca(2+) exchanger: molecular partners in pacemaker regulation. Circ Res 88: 1254-1258.
15. Hu ST, Liu GS, Shen YF, Wang YL, Tang Y, et al. (2011) Defective Ca(2+) handling proteins regulation during heart failure. Physiol Res 60: 27-37.
16. Martos R, Baugh J, Ledwidge M, O'Loughlin C, Conlon C, et al. (2007) Diastolic heart failure: evidence of increased myocardial collagen turnover linked to diastolic dysfunction. Circulation 115: 888-895.
17. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, et al. (2008) Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. J Am Coll Cardiol 52: 1574-1580.
18. de Jong S, van Veen TA, van Rijen HV, de Bakker JM (2011) Fibrosis and cardiac arrhythmias. J Cardiovasc Pharmacol 57: 630-638.
19. Horn MA, Graham HK, Richards MA, Clarke JD, Greensmith DJ, et al. (2012) Age-related divergent remodeling of the cardiac extracellular matrix in heart failure: collagen accumulation in the young and loss in the aged. J Mol Cell Cardiol 53: 82-90.
20. Yanni J, Tellez JO, Sutyagin PV, Boyett MR, Dobrzynski H (2010) Structural remodelling of the sinoatrial node in obese old rats. J Mol Cell Cardiol 48: 653-662.
21. Bakker ML, Boukens BJ, Mommersteeg MT, Brons JF, Wakker V, et al. (2008) Transcription factor Tbx3 is required for the specification of the atrioventricular conduction system. Circ Res 102: 1340-1349.
22. Hoogaars WM, Engel A, Brons JF, Verkerk AO, de Lange FJ, et al. (2007) Tbx3 controls the sinoatrial node gene program and imposes pacemaker function on the atria. Genes Dev 21: 1098-1112.
23. Borlak J, Thum T (2003) Hallmarks of ion channel gene expression in end-stage heart failure. FASEB 17: 1592-1608.
24. Joyner RW, van Capelle FJ (1986) Propagation through electrically coupled cells. How a small SA node drives a large atrium. Biophys J 50: 1157-1164.
25. Davis LM, Rodefeld ME, Green K, Beyer EC, Saffitz JE (1995) Gap junction protein phenotypes of the human heart and conduction system. J Cardiovasc Electrophysiol 6: 813-822.
26. Glukhov AV, Fedorov VV, Kalish PW, Ravikumar VK, Lou Q, et al. (2012) Conduction remodeling in human end-stage nonischemic left ventricular cardiomyopathy. Circulation 125: 1835-1847.
27. Kostin S, Rieger M, Dammer S, Hein S, Richter M, et al. (2003) Gap junction remodeling and altered connexin43 expression in the failing human heart. Mol Cell Biochem 242: 135-144.
28. Chaldoupi SM, Loh P, Hauer RN, de Bakker JM, van Rijen HV (2009) The role of connexin40 in atrial fibrillation. Cardiovasc Res 84: 15-23.
29. Burstein B, Comtois P, Michael G, Nishida K, Villeneuve L, et al. (2009) Changes in connexin expression and the atrial fibrillation substrate in congestive heart failure. Circ Res 105: 1213-1222.
30. Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, et al. (2007) Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. J Physiol 582: 675-693.
31. Nattel S (1999) Ionic determinants of atrial fibrillation and Ca2+ channel abnormalities : cause, consequence, or innocent bystander? Circ Res 85: 473-476.
32. Yue L, Melnyk P, Gaspo R, Wang Z, Nattel S (1999) Molecular mechanisms underlying ionic remodeling in a dog model of atrial fibrillation. Circ Res 84: 776-784.
33. Mangoni ME, Couette B, Bourinet E, Platzer J, Reimer D, et al. (2003) Functional role of L-type Cav1.3 Ca2+ channels in cardiac pacemaker activity. Proc Natl Acad Sci U S A 100: 5543-5548.
34. Matthes J, Yildirim L, Wietzorrek G, Reimer D, Striessnig J, et al. (2004) Disturbed atrio-ventricular conduction and normal contractile function in isolated hearts from Cav1.3-knockout mice. Naunyn Schmiedebergs Arch Pharmacol 369: 554-562.
35. Hagiwara N, Irisawa H, Kameyama M (1988) Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol 395: 233-253.
36. Irisawa H, Brown HF, Giles W (1993) Cardiac pacemaking in the sinoatrial node. Physiol Rev 73: 197-227.
37. Choy AM, Kuperschmidt S, Lang CC, Pierson RN, Roden DM (1996) Regional expression of HERG and KvLQT1 in heart failure. Circulation 94: 164.
38. Tsuji Y, Opthof T, Kamiya K, Yasui K, Liu W, et al. (2000) Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. Cardiovasc Res 48: 300-309.
39. Wang Z, Yue L, White M, Pelletier G, Nattel S (1998) Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. Circulation 98: 2422-2428.
40. Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, et al. (1995) Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. Circulation 92: 778-784.
41. Pye MP, Cobbe SM (1992) Mechanisms of ventricular arrhythmias in cardiac failure and hypertrophy. Cardiovasc Res 26: 740-750.
42. Armoundas AA, Hobai IA, Tomaselli GF, Winslow RL, O'Rourke B (2003) Role of sodium-calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts. Circ Res 93: 46-53.
43. Studer R, Reinecke H, Bilger J, Eschenhagen T, Böhm M, et al. (1994) Gene expression of the cardiac Na(+)-Ca2+ exchanger in end-stage human heart failure. Circ Res 75: 443-453.