HCN and SCN5A Channel Mutations: Implications for Impaired Atrioventricular Nodal Conduction in a Heterogeneous Computer Model of the Whole Mouse Heart

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Abstract

The atrioventricular (AV) node is a specialised region of the cardiac conduction system, allowing electrical excitation to propagate from the atria to the ventricles. AV block occurs when the AV node prevents electrical excitation waves reaching the ventricles, ultimately leading to a decrease in cardiac output. HCN4 and SCN5A defects have been associated with AV block, but the mechanisms underlying impaired AV node conduction remain incompletely understood.

In this study we constructed a one-dimensional model for the mouse whole heart featuring detailed single cell models for sino-atrial node, atrium, ventricle and the AV node. The functional effects of HCN4 knock-out and the D1595N (SCN5A) mutation were simulated by modifying ion channel properties of I_f and I_{Na} respectively.

Complete block of I_f in the model caused bradycardia; however, propagation was unaltered with no change in conduction velocity, suggesting the AV block caused by HCN4 knock-out is not a result of reduced I_f density. The D1595N mutation reduced conduction velocity throughout the model, leading to an increased PR interval (63ms vs. 43ms in control) indicative of 1st degree AV node block. The AV node exhibited reduced excitability which led to 2:1 block at a faster pacing rate.

1. Introduction

The atrio-ventricular (AV) node is a vital part of the cardiac conduction system, providing the only electrical conduction pathway between the atria and the ventricles. Propagation of the action potential (AP) is slower in the AV node than the working myocardium of the atria and ventricles, resulting in a delay in conduction which allows time for the ventricles to fill with blood following atrial contraction, before the ventricles contract themselves [1]. Furthermore, the AV node exhibits automaticity and can act as a subsidiary pacemaker when the sino-atrial node

(SAN) fails. However, the pacemaking activity of the AV node is suppressed under normal sinus rhythm, due to an intrinsically slower spontaneous pacing rate than that of the SAN [1].

Heart block, or AV node block, occurs when the conduction through the AV node is impaired or abolished. On the electrocardiogram (ECG), AV block is evident from examining the relationship between the P waves and QRS complexes, which correspond to activation of the atria and ventricles respectively.

The severity of AV node block varies according to the extent to which conduction through the AV node is delayed. There is an increased PR interval (interval between the P wave and R wave) associated with 1st degree block, indicating a delay in atrio-ventricular conduction. In 2nd degree heart block the conduction across the AV node is delayed to such an extent that some atrial APs fail to propagate to the ventricles, resulting in dropped QRS complexes on the ECG. In 3rd degree block the AV node does not allow any conduction to the ventricles, and QRS complexes show no correlation with P waves [1]. Ventricular activation may be sustained by subsidiary pacemaking cells within the His-Purkinje system [2], but usually a pacemaker is implanted to maintain the pumping action of the ventricles.

AV node block is commonly acquired i.e. following an event or events which cause structural and electrophysiological remodelling, such as heart failure. However, in some cases the cause can be congenital and related to ion channel mutation. Wang *et al*. linked two SCN5A mutations (G298S and D1595N) directly to AV node block [3]; both mutations result in an impaired sodium current affecting conduction velocity (CV) in tissue and excitability of cells.

The funny current also plays an important role in the cardiac conduction system; Baruscotti *et al*. showed that in a mouse model of HCN4 knock-out the funny current was down-regulated by 75-90%, eventually leading to complete AV node block and death [4]. The mechanism linking HCN4 knock-out remains to be determined;

whilst it is possible other channels may be remodelled over the course of the HCN4 knock-out [4], this result may also point to an unexpected role of I_f in conduction defects.

So far the mechanisms linking I_{Na} and I_f dysfunction to AV node block remain poorly understood. In this study we present a one-dimensional whole heart model of mouse electrophysiology as a platform for studying such defects. Our aims were two-fold:

1) To investigate the effect of SCN5A mutations on cardiac conduction in the whole heart, and how they relate to AV node block;
2) To evaluate the impact of I_f on AV node function and assess its role in cardiac conduction defects.

2. Methods

2.1. Single cell models

In this study we used a family of single cell models coupled together in a 1D strand. For the SAN and ventricular part of the strand, we used the Kharche *et al.* model [5] and Bondarenko model [6] respectively. In a separate study a model has been created for atrial cardiomyocytes based on the Bondarenko formulation [6], which was used in this study to simulate atrial electrophysiology.

In this study we have developed a model for mouse AV node based on modifications to the Kharche *et al.* model [5] for SAN. In order to simulate the differences in electrophysiology between the AV node and SAN we incorporated experimentally observed differences in mRNA expression [7] and fitted current and I-V traces to existing patch clamp data [8,9].

2.2. Genetic mutation

HCN4 knock-out (HCN4-KO) is characterised by a large reduction in the funny current [4]. Therefore, in order to simulate HCN4-KO we blocked I_f by 100% so as to investigate the worst possible effect.

Typically the kinetics of I_{Na} can be described by considering separate fast and slow inactivation. The two SCN5A mutations G298S and D1595N have similar effects on I_{Na} function, with both exhibiting impaired fast and slow inactivation. Wang *et al.* showed that fast inactivation is slowed, and that there is also a delayed recovery from slow inactivation [3].

In our simulations we modelled the effects of D1595N so as to investigate the worst case scenario, as this exhibited more severe effects on I_{Na} function. In order to replicate the effects of D1595N we multiplied the time constant of fast inactivation by a factor of 2, and to simulate delayed recovery from slow inactivation we negatively shifted the voltage dependence of the time constant for slow inactivation. The effects of these mutations were verified by simulating a two-pulse protocol to assess the availability of sodium channels (as in [3]).

2.3. One-dimensional strand

The models described above were coupled into a 1D strand in order to examine the effect of genetic mutation on conduction in the whole heart. The cable equation was solved to provide electrotonic coupling between cells.

The dimensions of the model were approximated based on experimental tissue preparations. The diffusion coefficient $D(x)$ was varied along the strand to replicate experimentally observed conduction velocities and intervals in each region (see table 1).

A gradient model of the SAN was incorporated following the approach of Zhang *et al.* [10] to reflect differences between central and peripheral SAN cells.

A pseudo-ECG was calculated [11] in order to assess the effect of HCN4-KO and SCN5A mutations on PP, RR and PR intervals.

3. Results

3.1. Single cell simulation

The AV node model exhibited a cycle length approximately 50% longer than the SAN, as experimentally observed [8]. A major feature of the AV node is its reduction in I_f channel density [6,7] compared to the SAN, which, at least in part, accounts for the reduction in cycle length.

We assessed the role of I_f in SAN and AV node by 100% block in both models. Interestingly we found that the increase in cycle length was more pronounced in AVN, which has been observed experimentally [4].
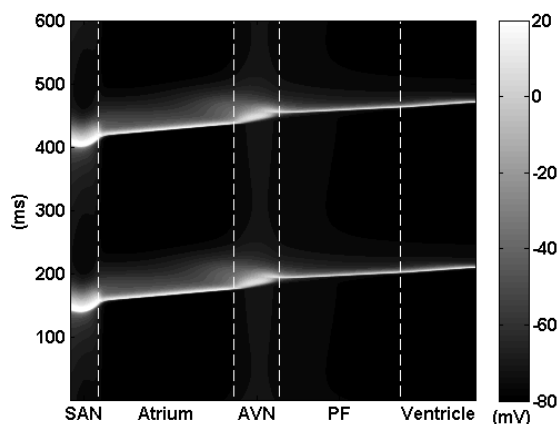

Figure 1. Spontaneously pacing 1D whole heart model during normal sinus rhythm.

3.2. Normal sinus rhythm

The 1-D whole heart model spontaneously paced from the SAN with a PP and PR interval of 262ms and 43ms respectively, as shown in Figures 1 and 2. The model also exhibited conduction velocities and intervals similar to those observed experimentally as detailed in table 1.

	Simulation	Experiment	Ref.
Atrial CV (m/s)	0.48	0.51 ± 0.004	[12]
AH interval (ms)	36.9	36 ± 5	[13]
HV interval (ms)	8.6	9.3 ± 1.4	[14]
Ventricular CV (m/s)	0.58	0.6 ± 0.07	[15]

Table 1. Comparison of conduction velocities (CV) and intervals for simulated and experimental data. References for experimental data are provided in the far right column. AH = atrio-Hisian interval; HV = His-ventricular interval.

3.3. Effect of HCN4-KO

The effect of blocking I_f in the 1D model was to increase the cycle length by 31% (PP = 344ms vs. PP = 262ms control). The PR interval showed no difference to the control value of 43ms; similarly, conduction velocities throughout the model were unaffected by block of I_f. These results suggest the bradycardia induced by HCN4-KO is largely due to the reduction of I_f, but the fact that no conduction defects are observed suggests AV block are not due to a reduction in I_f. As mentioned in [4] there may be additional ion channel remodeling during progressive HCN4-KO which would account for AV node block.
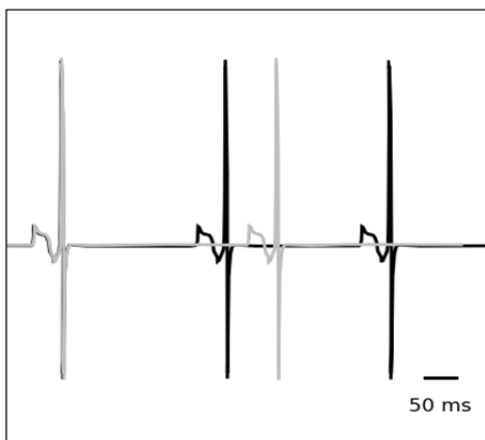

Figure 2. Calculated pseudo-ECG during normal sinus rhythm for control (black) and HCN4-KO (grey) simulations. The PP and RR intervals are increased due to HCN4-KO whereas the PR interval shows no difference compared to normal sinus rhythm.

3.4. Effect of D1595N

The D1595N mutation caused an increase in cycle length (PP = 330ms vs. PP = 262ms control). The PR interval was also increased by 47% (PR = 63ms vs. PR = 43ms control), which is indicative of 1st degree AV node block.

CV was reduced in the AV node by 53% (CV = 0.08m/s vs. CV = 0.18m/s control) and by 14% in the atrium (CV = 0.41m/s vs CV = 0.48m/s control). This indicates that the lengthening in PR interval is predominantly due to slowed CV in the AV node.

3.5. Rate dependence of AV nodal conduction

In order to further assess the effect of genetic mutation, we replaced the spontaneous pacing of the SAN with a manual stimulus at a faster basic cycle length (BCL) of 200ms.
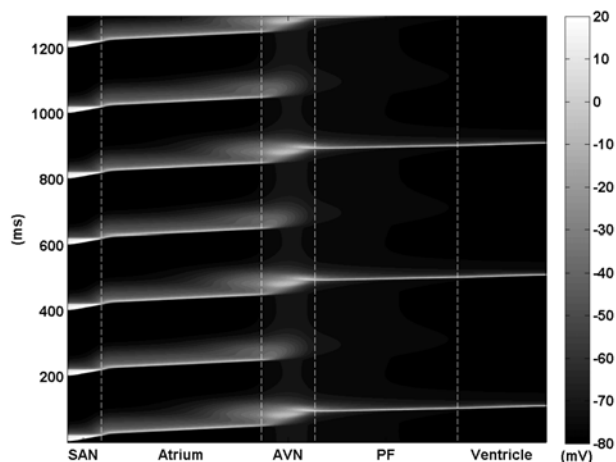

Figure 3. AP propagation in D1595N simulation at a BCL of 200ms. Atrial and ventricular beats exhibit a 2:1 ratio with alternate atrial beats failing to propagate via the AV node.

CV (m/s)	Control	HCN4-KO	D1595N
Atrium	0.47	0.47	0.39
AV node	0.16	0.16	0.07
Ventricle	0.60	0.60	0.50

Table 2. Comparison of conduction velocities (CVs) for control, HCN4-KO and D1595N mutation.

In control and HCN4-KO simulations, APs were successfully conducted from the atrial segment to the ventricular segment (data not shown). Conduction velocities were unaltered by the block of I_f.

Introduction of the D1595N mutation resulted in a 2nd

degree AV node block, with P waves and QRS complexes exhibiting a 2:1 ratio. Inspection of the AV nodal AP waveform revealed that the sodium current failed to elicit an AP during alternate beats. This can be attributed to the delayed recovery of slow inactivation of the sodium current.

4. Discussion

I_f plays an important role in the cardiac conduction system, having a huge impact on automaticity in the SAN [4] and AVN [8]. In our simulation we found block of I_f increased cycle length, reinforcing the role of the funny current in regulating heart rate. That said, we found no evidence to suggest that I_f is involved in AV node block, or any type of conduction disease.

The effect of SCN5A mutation was more pronounced showing 1st degree block during spontaneous pacing, and 2nd degree block when stimulating with a higher pacing rate. Due to the impaired recovery of I_{Na} from inactivation, the availability of sodium channels is reduced between beats and thus excitability is reduced. This also explains the reduced CV throughout the whole heart model.

5. Conclusion

In this study we have presented a whole heart model of mouse electrophysiology as a platform for studying cardiac disease. The model shows that I_f plays a role in regulating heart rate as expected, but our results suggest that there are no adverse affects to conduction due to blocking I_f. We find that the SCN5A genetic mutation D1595N slows CV resulting in a longer PR interval during spontaneous pacing, and 2nd degree AV node block during faster pacing.

Acknowledgements

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