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Effects of Elevated Homocysteine Hormone on Electrical Activity in the Human Atrium: A Simulation Study

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Abstract—**Atrial fibrillation (AF) accounts for a large proportion of healthcare expenditure world wide. Mechanisms underlying the genesis and maintenance of AF are still poorly understood. Though AF is largely thought to be caused and perpetuated by dysfunctions of cellular ion channels, disrupted intercellular gap junctional electrical coupling, and/or structural changes in the atria, it is also associated with abnormal secretion of hormones, such as a high level of Homocysteine (Hcy). It was found that a high concentration Hcy induces electrical remodeling of ion channels in human atrial cells that include the ultra rapid potassium, inward rectifier potassium and transient outward potassium currents. Such Hcy-induced ion channel remodeling in repolarising potassium currents has been hypothesized to be pro-arrhythmic. In this study, we carried out multi-scale simulations to evaluate the effects of Hcy-induced changes in potassium currents on the electrical activity of human atrium at single cell, 1D strand of tissue, and 3D anatomical models. We found that high Hcy concentration produced marked changes in atrial action potentials, including a more hyperpolarized resting potential, elevated plateau potential during early stages of repolarization and abbreviated action potential duration (APD). Losses in rate dependent accommodation of APD and effective refractory period were observed. In the tissue models, high Hcy concentration slowed down atrial excitation conduction at low rates, but facilitated it at high rates. Simulated re-entrant scroll waves in the 3D model self-terminated under Control condition, but sustained under high Hcy condition. These results collectively demonstrate the pro-arrhythmic effects of a high level Hcy in promoting and sustaining AF.**

I. INTRODUCTION

Atrial fibrillation (AF) is a degenerative disease affecting the upper chambers of mammalian hearts. AF is debilitating and requires extensive treatment [1, 2]. Although clinically well recognized, the underlying mechanisms of AF are not well understood. AF is thought to be perpetuated by structural degeneration of atrial tissue and changes in the electrophysiological properties of atrial cell ion channels. It has previously been shown that a decrease in the depolarizing currents or an abnormal increase in the repolarising currents at cellular level can lead to erratic wave propagation in spatially extended human atria resulting atrial fibrillation [3, 4, 5]. Along with structural and electrophysiological changes of atrial tissue, age related loss of gap junctional coupling has also been implicated as a contributor to self-perpetuating AF [6].

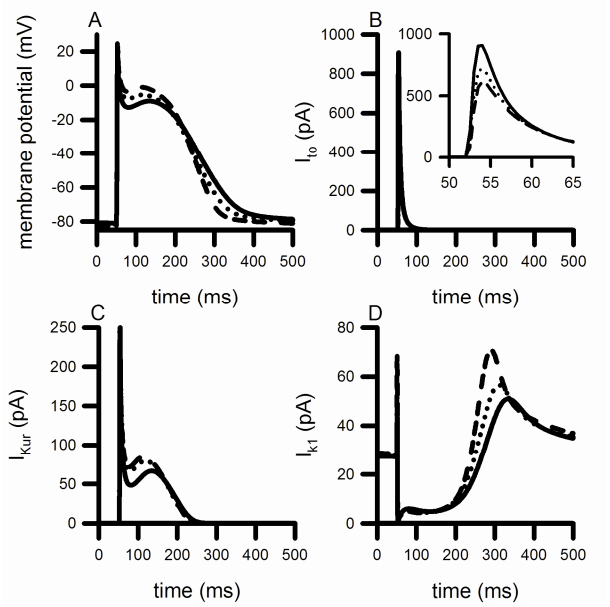

Fig. 1. Time traces of computed APs and some associated ionic currents under Control (solid lines), 50 μmol/L Hcy (dotted lines) and 500 μmol/L Hcy (dashed lines) conditions. A: AP profiles. B: I_{to}. C: $I_{K,ur}$. D: I_{K1}. Increased Hcy concentration results in APD_{90} abbreviation, more hyperpolarized resting potential, and elevated plateau-potential in the early repolarisation phase.

Abnormal secretion of hormones can also be pro-arrhythmic. Recent studies have shown that an increase in Homocysteine (Hcy) above physiological concentration levels (5 – 20 μmol/L) are important risk factors responsible for developing human heart diseases that include AF [7]. Further study has revealed that a high concentration of Hcy induces electrical remodeling in some repolarising potassium currents, including a down-regulation of the ultra-rapid potassium current, $I_{K,ur}$, and transient outward potassium current I_{to}, and up-regulation of the inward rectifier potassium current I_{K1} [8]. As these potassium currents play an important role in atrial repolarisation, alteration to them produce an unbalance between the depolarization and repolarization currents, which may lead

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to pro-fibrillatory disturbances in atrial excitation wave conduction propagation. However, the pro-arrhythmic effects of the Hcy-induced electrical remodeling on potassium current have not yet been substantiated explicitly.

In this study, we investigated the effects of an elevated level of Hcy hormone on atrial electrical activities by using biophysically detailed computer models of human atria at multi-scale levels that include cellular, 1D tissue and 3D anatomical organ model of human atria.

II. MATERIALS AND METHODS

The biophysically detailed model for the electrical action potential (AP) of human atrial cells developed by Courtemanche *et al.* [8] was implemented in our simulations. The model is well established and has been used in previous simulation studies [3, 4, 5, 10, 12]. The model was modified to incorporate experimental data of Cai *et* al. [8] on Hcy-induced electrical remodeling of several potassium ion channels. It was found that Hcy augments the I_{K1} conductance (g_{K1}), while reduces the I_{Kur} conductance ($g_{K,ur}$) and the I_{to} conductance (g_{to}). However, Hcy does not affect the kinetics of these channels. Based on the experimental data [8], the does-dependent alterations to the maximal channel conductance of g_{K1}, $g_{K,ur}$ and g_{to} in the Courtemanche *et al.* model [9] are summarized in Table 1.

TABLE I
CONDUCTANCE GAINS FOR POTASSIUM CHANNELS DUE TO HCY

Model	g_{to}	$g_{K,ur}$	g_{K1}
50 μmol/L	24.8% ↓	10% ↓	10% ↑
500 μmol/L	38.4% ↓	29.6% ↓	39.2% ↑

The original and modified Courtemanche *et al*. models were used to simulate atrial action potentials under Control and the Hcy conditions. APs were evoked by a series of 10 conditioning supra-threshold stimuli with a pacing cycle length (PCL) of 1000 ms, strength of 2 nA/pF and duration 2 ms each. Such conditioning stimulus was deemed to be sufficient to allow elicitation of stable AP profiles. The 10th AP was therefore noted for further analyses of the effects of Hcy on human atrial electrical excitation. APD_{50} was defined as the time interval taken from the 10th stimulus to the time when the evoked AP reached 50% repolarization. APD_{90} was defined as the time interval taken from the 10th stimulus to the time when the evoked AP reached 90% repolarization. APD_{90} restitution (APDr,90) and APD_{50} restitution (APDr,50) were computed using a standard S1-S2 protocol where a premature stimulus (S2) was applied at a time after the 10th conditioning stimulus of S1 (at a PCL of 1000 ms) as used in a previous study [10]. Diastolic interval (DI) was defined as the time interval between 90% repolarization of the previous AP and the upstroke of the final AP. A plot of measured APD_{50} and APD_{90} against DI gave APDr curves. Maximal slopes of the curves were determined. Effective refractory period (ERP) was defined as the minimum S1-S1 stimulus interval that produced an AP with peak potential over 80% of that of the final S1-evoked AP [11]. ERP was calculated over a range of PCLs and ERP restitution (ERPr) curve was constructed. Rate-dependent effects of Hcy on cellular electrical APs were further investigated by varying the PCL of the 10 conditioning stimuli at 1 Hz, 2 Hz and 4Hz.

The cell models were incorporated into a diffusion parabolic partial differential equation (PDE) to construct mono-domain models of spatially extended atrial tissue. The PDE has the form

$$C_m \partial u / \partial t = D\nabla^2 u - I_{ion} \quad (1)$$

Where C_m is cell membrane capacitance, D is the diffusive coefficient modeling the intercellular electrical coupling of cells due to the gap junctional coupling, u is the membrane potential and I_{ion} is the total membrane current. In the model, D was set to 0.031 mm^2/ms that produces a physiological conduction velocity (CV) of 0.27 m/s for a solitary excitation wave under Control condition [4].

In the 1D strand model, the inter-cellular distance, represented by the spatial resolution of the model, was taken to be 0.1 mm. Such a space step is close to the length of human atrial cells and is sufficiently small enough to give stable numerical solutions of the model. The 1D strand model of homogenous atrial fiber has a length 20 mm, which is discretized to form 200 coupled cells. Using the 1D model, conduction velocity restitution (CVr) and temporal vulnerable window (VW) of atrial tissue to uni-directional conduction block were computed using the methods as described in a previous study [4].

Anatomically detailed 3D model of human atria was developed in our previous study [12]. The 3D model has a spatial resolution of 0.33 mm × 0.33 mm × 0.33mm. The model was numerically solved by the explicit Euler method with a time step 0.005 ms, which guarantees sufficiently accurate solutions [13]. Using similar stimulation protocols as used in a previous study [13], reentrant excitation scroll waves were initiated for both Control and elevated Hcy (500 μmol/L) conditions.

III. RESULTS

The simulated APs under Control and Hcy (500 μmol/L) conditions at a PCL of 1000 ms are show in Fig. 1. Hcy alters the morphology of the AP substantially. These changes included an abbreviated APD (both the APD_{50} and APD_{90}), elevated plateau potential during the early stage of repolarisation and a more hyperpolarized resting potential. The altered AP profile can be attributable to the integrative action of the Hcy-induced electrical remodeling of the I_{to}

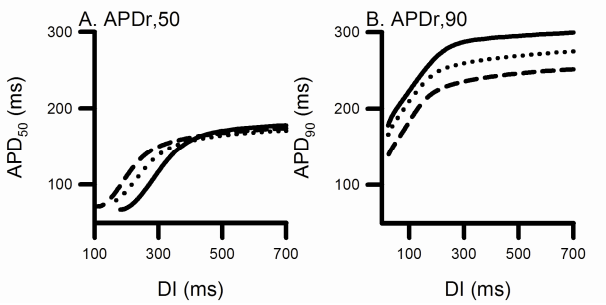

Fig. 2. Computed APDr,50 (A) and APDr,90 (B) under Control (solid lines), 50 µmol/L (dotted lines) and 500 µmol/L (dashed lines) conditions. APDr,50 shows that APD_{50} is shortened by Hcy at low excitation rates, but prolonged by Hcy at high excitation rates. APDr,90 shows that APD_{90} is consistently abbreviated by Hcy conditions.

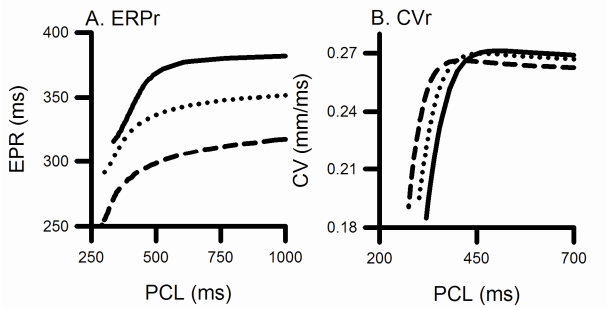

Fig. 3. Computed ERPr (A) and CVr (B) under Control (solid line), 50 µmol/L (dotted line) and 500 µmol/L (dashed line) Hcy conditions. Hcy reduces ERP and flattens its restitution curve. Shift of the ERPr curve left-ward indicates the loss of rate-dependent accommodation of ERP, which is pro-arrhythmic. Hcy reduces atrial tissue's excitability resulting in slowed intra-atrial conduction. Shift of the CVr curve left-ward indicates that Hcy facilitates atrial tissue to conduct high rate excitation waves.

(Fig. 1B), $I_{K,ur}$ (Fig. 1C) and I_{K1} (Fig. 1D). As Hcy decreases I_{to}, which is responsible for the early stage of repolarisation, the plateau potential is elevated. Though the maximal channel conductance of $I_{K,ur}$ is reduced, but due to the elevated plateau potential, $I_{K,ur}$ is increased in the early phase of repolarisation, but decreased in successive repolarisation phases. Augmented I_{K1} contributes mainly to a more hyperpolarized resting potential and an abbreviated APD. At a PCL of 1000 ms, APD_{50} was moderately reduced by Hcy (50 µmol/L: 5.4% reduction; 500 µmol/L Hcy: 6.5% reduction) whilst APD_{90} was reduced to a larger extent (50 µmol/L: 7.7% reduction; 500 µmol/L: 16.2% reduction).

The effects of Hcy hormone on APD is rate dependent as shown in Fig.2. It shortens APD_{50} at low excitation rates (i.e., large DIs), but prolongs APD_{50} at high excitation rates (i..e, small DIs) (Fig. 2A). However, Hcy abbreviates APD_{90} consistently for both high and low excitation rates (Fig. 2B). For both the APDr,50 and APDr,90 curves, Hcy flattens them and shifts them left-ward, indicating the loss of rate dependent accommodation of APD, similar to the functional impacts of chronic AF-induced electrical remodeling on human atrial action potentials [11]. In addition, we found that a simple estimation of maximal slopes did not necessarily predict the behavior of re-entrant waves in spatial models.

Hcy reduces ERP immensely. In the Control condition, the measured ERP is 382.0 ms. However, in the Hcy conditions, the measured ERP decreases to 352.0 ms for 50µmol/L Hcy and 318.0 ms for 500 µmol/L Hcy at a PCL of 1000 ms. Hcy also flattens the ERP restitution curve and shifts it left-ward (Fig. 3A), indicating the loss of the rate dependent accommodation of ERP, similar to the observation of human atrial cells due to AF-induced electrical remodeling [11].

Hcy hyperpolarizes the resting potential of atrial cells, resulting in a decreased tissue's excitability. This is reflected by the slowing down in intra-atrial excitation wave conduction. Computed CV of solitary waves from the 1D tissue model decreases with increased concentrations of Hcy. Under the Control condition, with a PCL of 1000ms the measured CV is 0.27 m/s, which reduces to 0.26 m/s with Hcy 50 µmol/L and 0.25 m/s for Hcy 500 µmol/L.

CV is also rate dependent as shown by the computed CVr curve (Fig. 3B). At low excitation rates (i.e., large PCLs), Hcy decreases CV due to the reduced atrial tissue's excitability as a consequent of a more hyperpolarized resting potential. As Hcy hyperpolarizes the resting potential to a more negative voltage range, it requires more stimulus current to depolarize cell membrane potential from its resting state to the excitation threshold potential of the cell (normally determined by the activation potential of the sodium channel, ~ -65 mV), thus the tissue's excitability is decreased. However, at high excitation rate (i.e., small PCLs), Hcy increases CV due to the reduced APD and ERP, which allows tissue to recovery sooner from previous excitation.

Effects of Hcy on tissue's vulnerability to produce uni-directional conduction block by a premature stimulus applied to the refractory tail of a previous excitation wave is also computed. Hcy reduces slightly the measured VW, which decreases from 8.66 ms under Control condition to 8.39 ms under Hcy 50µmol/L and 7.64 ms under Hcy 500µmol/L.

Hcy increases the stability of reentrant excitation wave as shown in Fig. 4, in which snapshots of the 3D simulations are shown for Control and Hcy conditions. Under the Control condition, the scroll wave is unstable with its organization centre (i.e., filament) meandering in a large area of tissue. The scroll wave is non-sustainable as it self-terminates at approximately 4.1 s. The self-termination is due to a combination of factors such as longer wavelength (i.e., larger ERP and higher CV) and non-stationary, which allow the self-termination of re-entrant waves within the atrial tissue. However, under the Hcy condition (500 µmol/L), the simulated scroll wave sustains through the whole period of simulation (6 s). The increased persistence of reentrant excitation wave under Hcy condition is attributable to the shortened wavelength of excitation waves, as a result of abbreviated ERP and reduced CV.

Distributed memory (MPI) parallalisation was implemented in the spatial simulations, especially in the 2D and 3D cases with formalisms developed previously [13].

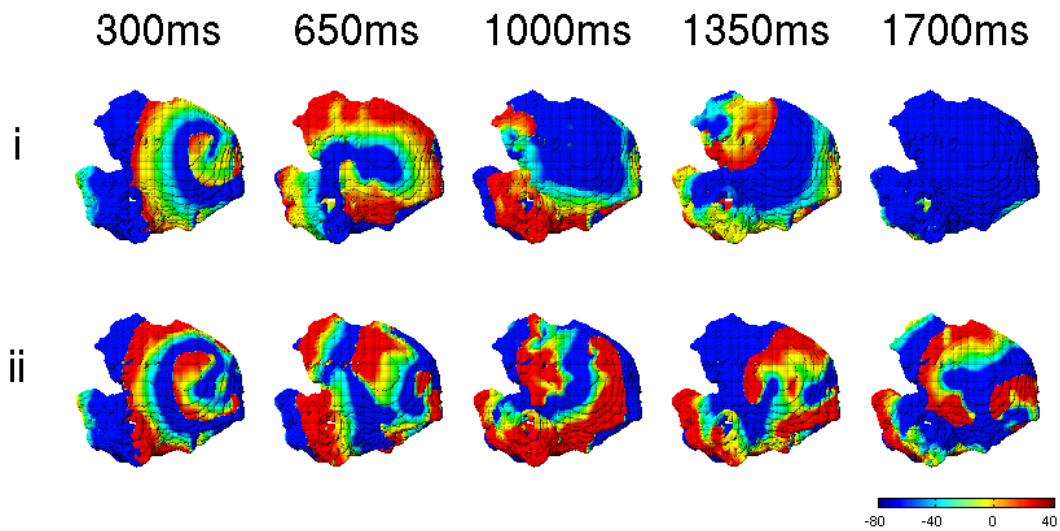

Fig. 4. Snapshots of propagation of re-entrant excitation waves in 3D anatomical model of human atria under Control (i) and Hcy 500µmol/L (ii) conditions. Under Control condition, scroll wave self-terminates at 1700ms. In the presence of Hcy, scroll wave persists for the full period of the simulation (6 s).

The 2D simulations took 2 hours using 10 processors whilst the 3D simulations took 6 hours on 32 processors on the local university HPC facility.

IV. Conclusions and Discussion

Many factors have been identified as contributors to AF genesis and perpetuation that include ageing, heart disease-induced electrical and structural remodeling, and gene mutations. While these have been extensively studied clinically and experimentally, little knowledge has been obtained about the role of abnormal secretion of hormones. In this study, we investigated the functional role of an elevated Hcy on atrial excitation by using biophysically detailed computer models. Our simulations show that the Hcy-induced electrical remodeling in I_{to}, $I_{K,ur}$ and I_{K1} currents have marked effects on the electrical activities of human atrial cells thereby affecting excitation wave propagation. The pro-arrythmogenic effects of an elevated Hcy are obvious with the reduction in the action potential duration, effective refractory period and intra-atrial conduction velocity, and losses of their rate-dependent accommodation. The integrative action of these cellular and tissue properties reduces the wavelength of atrial excitation waves allowing atria to sustain high rate reentrant excitation waves. This pro-arrhythmic mechanism is similar to that of AF-induced electrical remodeling on some ion channels [3, 11, 14] and gain-in-function of I_{K1} arising from KCNJ2 gene mutation [4]. This study substantiates the link between an elevated Hcy concentration and incidence of AF in some patients.

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