P73

Electrotonic effects on transmural differences in action potential duration: a computational study

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Transmural differences in action potential shape and duration (APD) have been identified in ventricular muscle (Antzelevitch *et al.* 2001), and these differences may be important in both the initiation and the evolution of re-entrant arrhythmias. The aim of this study was to use a simplified computational model of electrical activity in the ventricular wall to investigate the relative effects of electrotonic current flow and differences in cellular properties on transmural APD differences during the propagation of a normal beat.

Our simulated ventricular wall had cellular electrophysiology described by the Luo-Rudy phase 1 equations, with parameters modified as in Qu *et al.* (2000). The dimensions approximated a wedge from the canine LV free wall with dimensions of $4 \times 4 \times 10$ mm, and fibre rotation of 120 deg between endocardial and epicardial surfaces. Anisotropic conduction was produced by a diffusion tensor with components of $0.002\ \mathrm{cm^2\ ms^{-1}}$ parallel to fibres and $0.0005\ \mathrm{cm^2\ ms^{-1}}$ perpendicular to fibres, and these values gave plane wave conduction velocities of $0.81\ \mathrm{ms^{-1}}$ and $0.36\ \mathrm{ms^{-1}}$ respectively. Numerical solutions used an adaptive time step of between 10 and 100 μs, and a space step of 200 μm, with no-flux boundary conditions at each surface.

We simulated propagation of four normal beats by endocardial activation with a cycle length of 500 ms in each of two models. In the first model, the maximal K^+ conductance was set to 0.3 mS $\mathrm{cm^{-2}}$ throughout. In single cells paced at a cycle length of 500 ms this value gives an APD of 163 ms. In the second model we imposed a linear change in maximal K^+ conductance from 0.2 mS $\mathrm{cm^{-2}}$ at the endocardium (single cell APD of 189 ms), to 0.4 mS $\mathrm{cm^{-2}}$ at the epicardium (single cell APD of 144 ms).

For the first model with uniform maximal K^+ conductance, APD varied with transmural distance from 168 ms on the endocardial surface to 150 ms on the epicardial surface. The ratio (epi/endo) of these values is 0.89, which compares with a value of 0.83 reported from experimental studies (Antzelevitch, *et al.* 2001). For the second model with non-uniform maximal K^+ conductance slab, APD varied from 175 ms on the endocardial surface to 143 ms on the epicardial surface, giving the ratio 0.81. We suggest that during normal transmural propagation of the action potential, electrotonic current flow prolongs repolarisation of endocardial layers and shortens repolarisation of epicardial layers. Transmural differences in APD can potentially arise from electrotonic effects as well as differences in the expression of ion channels.

Antzelevitch C *et al.* (2001). *Phil Trans Roy Soc Lond A* **359**, 1201–1216.

Qu ZL *et al.* (2000). *Biophys J* **78**, 2761–2775.

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P74

Inhibition of Ca^{2+} current and twitch tension by divalent (ferrous) iron in isolated rat ventricular myocytes

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Serum iron can become elevated in a number of conditions (see e.g. Trinder *et al.* 2002), including acute iron poisoning, genetic disease (notably hereditary haemochromatosis, which affects around 1 in 300 people in Caucasian populations) and repeated blood transfusions. Iron overload is associated with both cardiac fibrosis and an increased risk of arrhythmias. However, the mechanisms by which iron causes these unwanted effects remain unclear. We have examined the effects of divalent (ferrous) iron (Fe^{2+}) on Ca^{2+} handling and mechanical activity in rat ventricular myocytes. Rats were humanely killed and cells were isolated by collagenase–protease digestion and loaded with the Ca^{2+}-sensitive fluorophores fura-2 or fluo-4, or the iron-sensitive fluorophore calcein. The cells were either field stimulated or voltage clamped in the amphotericin B-perforated patch configuration. In all experiments cells were superfused with Tyrode solution at room temperature and stimulated at a frequency of 0.5 Hz.

Iron-containing Tyrode solution contained 100 or 200 μM iron, together with 1–5 mM ascorbate to ensure that iron was in the reduced ferrous (+2) oxidation state. In field stimulation experiments, addition of Fe^{2+} reduced cell shortening and/or made cells refractory to stimulation. In voltage-clamp experiments, 100 or 200 μM Fe^{2+} caused a marked reduction in the amplitude of the Ca^{2+} current and a parallel reduction in the amount of cell shortening. Both effects were rapid and reversible. Interestingly, the reduction in Ca^{2+} current and shortening was not accompanied by any detectable changes in sarcoplasmic reticulum Ca^{2+} loading, suggesting that the decrease in trigger L-type Ca^{2+} current produced by Fe^{2+} was solely responsible for the decrease in shortening. Finally, we only rarely observed any irreversible effects of Fe^{2+} and ascorbate that might be ascribable to oxidant stress, although this might reflect the short duration of Fe^{2+} exposure and/or the relatively low concentration of Fe^{2+} used.

The effects of Fe^{2+} reported here are consistent with block of L-type Ca^{2+} channels by sub-millimolar concentrations of Fe^{2+}. This contrasts with a previous report by Tsushima *et al.* (1999) who found that Fe^{2+} inhibited L-type Ca^{2+} currents in ventricular myocytes only at concentrations of 2 mM or greater. The reason for this discrepancy is not known. Overall, our results suggest that, like other di- and trivalent metal cations, Fe^{2+} is an effective blocker of voltage-gated Ca^{2+} entry in the heart.

Trinder F *et al.* (2002). *Gut* **51**, 290–295.

Tsushima RG *et al.* (1999). *Circ Res* **84**, 1302–1209.

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All procedures accord with current UK legislation.

P75

Effects of pH on ventricular myocyte contraction and intracellular Ca^{2+} in streptozotocin-induced diabetic rats

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The concentration of acetoacetic acid, β-hydroxybutyric acid and acetone in blood may rise to very high levels in diabetic patients and cause metabolic acidosis. The aim of this study was to investigate the acute effects of acidosis on ventricular myocyte contraction and intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in streptozotocin (STZ)-induced diabetic rats.

Diabetes was induced in rats by I.P. injection of STZ (60 mg kg^{-1}). Experiments were performed in electrically stimulated (1 Hz) myocytes, maintained at 35–36 °C, from control and diabetic rats at 8–12 weeks after STZ treatment. Characteristics of shortening were measured with a video edge detector (VED-114, Crystal Biotech, USA). Intracellular $[Ca^{2+}]$ was measured in myocytes loaded with fura-2/AM. Electrically stimulated myocytes were exposed to normal Tyrode solution pH adjusted to either 7.4 (NT) or 6.4 (acid NT). Data are expressed as means ± S.E.M. of n observations. Statistical comparisons were performed using either an independent samples t test or one-way ANOVA followed by Bonferoni corrected t tests for multiple comparisons as appropriate. P values less than 0.05 were considered significant.

The general characteristics of STZ-induced diabetes included significant ($P < 0.05$) reductions in body and heart weight and a 5-fold increase in blood glucose. Time to peak shortening was significantly prolonged in myocytes from STZ-treated (131 ± 5 ms, $n = 14$) compared to control (109 ± 5 ms, $n = 20$) rats. Change of external pH from 7.4 to pH 6.4 significantly reduced the amplitude of shortening in electrically stimulated control and diabetic myocytes, but the magnitude of the negative inotropic effects of acid NT were not additionally altered by STZ-treatment. Time to peak shortening and to half relaxation in control and diabetic myocytes were not altered by exposure to acid NT. The amplitude of the Ca^{2+} transient was significantly reduced by acid NT but the magnitude of the response was not additionally altered by STZ-treatment. The acute effects of exposure to acid NT on myocyte shortening and Ca^{2+} transient were not significantly altered by STZ-induced diabetes.

Animals were killed humanely and all experimental procedures were carried out in accordance with internationally acceptable practices and were approved by Faculty of Medicine & Health Sciences, United Arab Emirates University Ethics Committee.

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All procedures accord with current local guidelines.

P76

Chronic β-blockade enhanced 5-HT-effects on action potentials in human atrial cells

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Long term treatment with β-adrenoceptor antagonists (β-blockers) has been associated with an enhanced 5-HT-induced increase in human atrial contractility (Sanders *et al.* 1995). However, the influence of chronic β-blockade on the human atrial cellular electrophysiological response to 5-HT has not yet been studied. We aimed to investigate the effect of 5-HT on action potentials and refractoriness in patients treated and not treated with β-blockers.

The whole cell perforated patch clamp technique was used to record electrical activity from cells isolated enzymatically from the right atrial appendage of consenting patients undergoing cardiac surgery (Workman *et al.* 2001). Procedures for obtaining tissues were approved by the institutional ethics committee, and conform to the Declaration of Helsinki. All patients were in sinus rhythm at the time of surgery.

The heart rate was significantly higher in patients not treated with β-blockers, at 78 ± 3 beats per minute (bpm) (mean ± S.E.M.) than in patients treated with β-blockers, at 63 ± 4 bpm ($P < 0.05$; Student's unpaired t test; $n = 11$ and 18 patients, respectively). In cells from the non-β-blocked patients, 5-HT produced a significant increase in the action potential duration at 50 % repolarisation, APD_{50} (measured at a constant pacing rate of 75 bpm), from 6 ± 1 to 17 ± 5 ms ($P < 0.05$, $n = 15$ cells, 7 patients). In these cells, 5-HT had no effect on the APD_{90} or the effective refractory period (ERP), at 190 ± 21 and 193 ± 24 ms ($n = 11$, 7 patients), in the absence of 5-HT, and 206 ± 26 and 213 ± 25 ms, in its presence, respectively. In the patients treated with β-blockers, 5-HT significantly increased the APD_{50}, from 12 ± 4 to 44 ± 9 ms ($P < 0.05$). In these cells, 5-HT had no effect on the APD_{90} or the ERP, at 236 ± 20 and 246 ± 19 ms, in the absence of 5-HT, and 224 ± 24 and 244 ± 18 ms, in its presence. In the cells from patients treated with β-blockers, the 5-HT-induced prolongation of APD_{50} was significantly greater (absolute prolongation: 32 ± 8 ms) than in the untreated patients (11 ± 5 ms; $P < 0.05$). Moreover, abnormal automaticity occurred in response to 5-HT, in 5 cells (4 patients) of the 21 cells (12 patients) studied in the patients treated with β-blockers but was not recorded in any of the cells from the patients who were not treated with β-blockers.

In conclusion, these data suggest that in the human atrium, chronic β-blockade was associated with a more pronounced effect of 5-HT to prolong early repolarisation and induce automaticity.

Sanders L *et al.* (1995). *Circulation* **92**, 2526–2539.

Workman AJ *et al.* (2001). *Cardiovasc Res* **52**, 226–235.

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P77

Collagen remodelling during the progression of left ventricular hypertrophy and heart failure

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Individuals suffering from hypertension and cardiac disease can exhibit myocardial hypertrophy and contractile dysfunction as well as a range of electrophysiological and histological changes within the myocardium including interstitial fibrosis, increased myocardial stiffness, decreased heart rate variability and prolonged QT interval. During the progression to cardiac failure, considerable remodelling of the myocardial extracellular matrix is known to occur, although it remains to be determined if these pathologies are due, in part, to increased collagen deposition/decreased collagen degradation, or conversely that changes in the ratio of type I to type III collagens are responsible (Weber, 1989). In this study, we have employed a model of left ventricular hypertrophy (LVH) and heart failure (HF) in the ferret in order to accurately relate changes in collagen turnover with the development of LVH and HF.

All procedures accorded with UK legislation (isoflurane inhalational anaesthesia for surgical procedures; Schedule 1 killing, pentobarbitone 200 mg kg^{-1}).

Heart failure occurred 8–12 weeks following ascending aortic coarctation, the mean heart/body weight ratio (± S.E.M.) increased from 4.7 ± 0.2 g kg^{-1} in sham operated animals, to 9.3 ± 0.5 g kg^{-1} in HF ($n = 17$, $P < 0.005$ Student's unpaired t test). ECG data gathered from a subset of animals using telemetry implants showed that the corrected RT interval (used as a measure of the QT interval in this model) increased during the development of LVH and HF by 17 ± 3 % of pre-operative values measured when clinical symptoms of HF were present ($n = 3$, $P < 0.005$, paired t test). Furthermore, heart rate variability (measured as the standard deviation of the mean heart rate from sequential 24 h periods) decreased with the onset of heart failure. No such change was observed in sham-operated animals.

Collagen levels in the left ventricular myocardium were determined when clinical symptoms of HF were present using a combination of high performance liquid chromatography (HPLC) and histological analysis. The HPLC data showed that in HF with LV chamber dilatation the mean collagen content per dry weight of tissue was reduced compared to sham operated animals (2.7 ± 0.4 % *vs.* 4.5 ± 0.5 %, $n = 6$, $P < 0.05$, unpaired t test). This decrease in the collagenous matrix of the myocardium may partly explain the observation of left ventricular chamber dilatation during heart failure. It still remains to be determined to what extent changes in myocardial collagen concentration influence the observed changes in heart rate variability and susceptibility to arrhythmias in this model of heart failure.

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P78

A 3D model of dolphin ECG biopotential distribution. The effect of seawater

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Diving in mammals is an exciting physiological challenge in that a number of cardiac and vascular reflexes are initiated to adjust the cardiac output to the reduced oxygen availability. However, quantification of electric cardiac activity during submergence in seawater is hampered by several technical problems, including signal attenuation, but also, for mammals other than man it is necessary to ascertain the optimal position for each electrode placement. To answer both questions we modelled a dolphin body, its heart, its skin and the surrounding seawater.

It is assumed that the heart polarization vector changes slowly so that electromagnetic effects can be disregarded and the electrodynamic problem can be solved as a succession of electrostatic problems. Under this assumption, each electropotential distribution can be obtained through the solution of the Laplace equation. The method chosen for the solution of Laplace's equations was the finite element method (FEM), which allowed the electrostatic field boundary conditions between dolphin body, skin and seawater, without the need of mathematical enforcement. In order to solve the numerical problem, the dolphin body and fins have been discretized and covered with a skin (Behrmann, 1997). Then, the discrete model was submerged in a seawater mesh. Finally, the model was completed by incorporation of tissue (muscle and skin) resistivities (Hyttinen *et al.* 1997). These values were measured in the laboratory with a four-point probe on dead specimens.

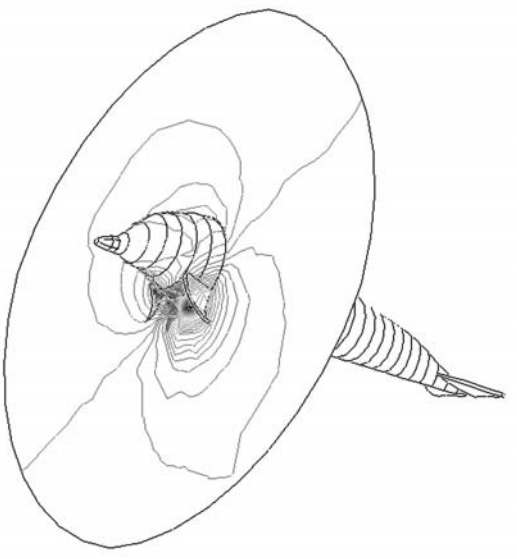

Figure 1. Distribution of isopotential lines for a modelled dolphin obtained in an instant near the QRS maximum.

Assuming that the heart acts as a dipole, the limit in the seawater around the dolphin was taken at a distance far enough to avoid the influence of boundary conditions in the electropotential distribution within the dolphin. The dipole intensity and direction within the dolphin heart is assumed from comparison

with the human heart. Care was taken with the ventricular repolarization, the T wave, which appears as a deep downward deflection in a dolphin ECG.

The simulated ECG, without the seawater, was validated by comparing the modelled ECG with the records obtained in captive dolphins, the results showing a very good agreement both in shape and intensity. After this validation, seawater was added to the model and its influence on the signal levels and distribution was analysed (Fig. 1).

Behrmann G (1997). *Additional Contribution to the Anatomy of Toothed Whales* (*Odontoceti*), Nordseemuseum, Bremerhaven Germany.

Hyttinen J *et al.* (1997). *Proc 19th Int Conf IEEE/EMBS*, 30/10-2/11.

All procedures accord with current national legislation.