Gap junction inhibition by heptanol increases ventricular arrhythmogenicity by decreasing conduction velocity without affecting repolarization properties or myocardial refractoriness in Langendorff-perfused mouse hearts

Running title: Gap junction inhibition, reduced conduction velocity and ventricular arrhythmogenesis in mice

Gary Tse MBBS MA PhD [1], Jie Ming Yeo BSc MBBS [2], Vivian Tse [3], Bing Sun MD PhD [4]

[1] School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, University of Hong Kong

[2] Faculty of Medicine, Imperial College London, SW7 2AZ UK

[3] Department of Physiology, McGill University, Canada H3G 1Y6

[4] Department of Cardiology, Tongji University Affiliated Tongji Hospital, Shanghai 200065, China

Keywords: conduction; conduction velocity; repolarization; refractory period; wavelength; gap junction; arrhythmia

Abstract

Arrhythmogenic effects of the gap junction inhibitor heptanol (0.05 mM) were examined in Langendorff-perfused mouse hearts. Monophasic action potential recordings were obtained from the left ventricular epicardium during right ventricular pacing. Regular activity was observed both before and after application of heptanol in all of the 12 hearts studied during 8 Hz pacing. By contrast, induced ventricular tachycardia (VT) was observed after heptanol treatment in 6 out of 12 hearts using a S1S2 protocol (Fisher's exact test, $P < 0.05$). The arrhythmogenic effects of heptanol were associated with increased activation latencies from 13.2 ± 0.6 to 19.4 ± 1.3 ms (ANOVA, $P < 0.001$) and reduced conduction velocities (CVs) from 0.23 ± 0.01 to 0.16 ± 0.01 ms (ANOVA, $P < 0.001$) in an absence of alterations in action potential durations at x = 90% (38.0 ± 1.0 vs. 38.3 ± 1.8 ms), 70% (16.8 ± 1.0 vs. 19.5 ± 0.9 ms), 50% (9.2 ± 0.8 vs. 10.1 ± 0.6 ms) or 30% (4.8 ± 0.5 vs. 6.3 ± 0.6 ms) repolarization (APD_x) or in effective refractory period (ERPs) (39.6 ± 1.9 vs. 40.6 ± 3.0 ms) (all $P > 0.05$). Consequently, excitation wavelengths (λ, CV x ERP) were reduced from 9.1 ± 0.6 to 6.5 ± 0.6 mm ($P < 0.01$) but critical intervals for re-excitation (APD_{90} – ERP) were unaltered (-1.1 ± 2.4 vs. -2.3 ± 1.8 ms; $P > 0.05$). Together, these findings demonstrate for the first time that inhibition of gap junctions alone using a low heptanol concentration (0.05 mM) was able to reduce CV, which alone was sufficient to permit the induction of VT using premature stimulation by decreasing λ, which therefore appears central in the determination of arrhythmic tendency.

Introduction

An orderly spread of action potentials through the heart is critical for normal electrical function and its disruption can lead to cardiac arrhythmias (1). Experiments in pre-clinical models has advanced our understanding of the electrophysiological mechanisms underlying arrhythmogenesis using genetic and pharmacological approaches (2-26). Experiments in mouse systems have shed light on the role of gap junctions in ventricular conduction and arrhythmogenesis, but have also led to some disagreements. Heterozygous $Cx43^{+/-}$ mice showed 45 to 50% decrease in Cx43 expression, but the degree of conduction velocity (CV) slowing was variable. CV was either unchanged (27-32) or decreased by 23 to 44% (33-35). Other experiments used a pharmacological approach, demonstrating ventricular arrhythmogenesis associated with decreased CV using 2 mM heptanol (7). This agent inhibits gap junctions specifically at concentrations up to 1 to 2 mM (36, 37) but at $\geq$ 2 mM also inhibits sodium channels (36, 38). It was unclear the extent to which the conduction defects and arrhythmogenesis observed could be attributed to loss of gap junction coupling alone.

Therefore, the aims of the present experiments are to examine the possible role of abnormal gap junction function in ventricular arrhythmogenesis, by applying heptanol at a low concentration that specifically targets gap junctions (0.05 mM). At this concentration, it was found that heptanol did not elicit spontaneous arrhythmias during regular pacing, but increased the incidence of ventricular tachycardia induced by a S1S2 protocol. This was associated with increased activation latencies in an absence of alterations in either action potential durations or effective refractory periods. The present findings suggest that loss of gap junction function alone was sufficient to produce ventricular arrhythmogenesis.

Materials and Methods

Solutions

The experiments described in this study used Krebs-Henseleit solution (composition in mM: NaCl 119, $NaHCO_3$ 25, KCl 4, KH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10 and sodium pyruvate 2, pH 7.4) that had been bicarbonate-buffered and bubbled with 95% O_2–5% CO_2 (39). Heptanol (Sigma, Dorset, UK; density: 0.82 g ml^{-1}) is soluble in aqueous solutions up to 9 mM (The Merck Index, New Jersey, USA), and was diluted using Krebs-Henseleit solution to produce a final concentration of 0.05 mM.

Preparation of Langendorff-perfused mouse hearts

Wild-type mice of 129 genetic background between 5 and 7 months of age were used in this study. They were maintained at room temperature (21 ± 1°C) amd subject to a 12:12 h light / dark cycle with free access to sterile rodent chow and water in an animal facility. The experiments described here were compliant with the UK Animals (Scientific Procedures) Act 1986. The procedures for the preparation of Langendorff-perfused mouse hearts are as follows. Mice were killed by cervical dislocation in accordance with Sections 1(c) and 2 of Schedule 1 of the UK Animals (Scientific Procedures) Act 1986. The hearts were rapidly excised and immediately submerged in ice-cold Krebs-Henseleit solution. Cannulation of the aorta was achieved using a tailor-made 21-gauge cannula that had been prefilled with ice-cold buffer. Using a micro-aneurysm clip (Harvard Apparatus, UK), the heart was securely attached to the perfusion system. Retrograde perfusion was initiated at a rate of 2–2.5 ml min^{-1} using a peristaltic pump (Watson–Marlow Bredel pumps model 505S, Falmouth, Cornwall, UK) with the perfusate passing through 200 and 5 μm filters successively and heated to 37°C using a water jacket and circulator before reaching the aorta. The hearts that regained their pink colour and spontaneous rhythmic activity were studied further (approximately 90%).

The remaining 10% were discarded. Perfusion took place for a further 20 minutes to minimise any residual effects of catecholamine released endogenously, before electrophysiology of the hearts was studied.

Stimulation protocols

Electrical stimulation was achieved using paired platinum electrodes (1 mm interpole distance) placed at the right ventricular epicardium. This took place at 8 Hz, using square wave pulses 2 ms in duration, with a stimulation voltage set to three times the diastolic threshold (Grass S48 Stimulator, Grass-Telefactor, Slough, UK) immediately after the start of perfusion. The S1S2 protocol was used to assess arrhythmogenicity and identify re-entrant substrates. This consisted of a drive train of eight regularly paced S1 stimuli separated by a 125 ms basic cycle length (BCL), followed by premature S2 extra-stimuli every ninth stimulus. The S1S2 interval was first set to 125 ms and then successively reduced by 1 ms with each nine stimulus cycle until arrhythmic activity was initiated or refractoriness was reached, whereupon the S2 stimulus elicited no ventricular response.

Recording procedures

Monophasic action potentials (MAPs) recordings from the left ventricular epicardium were obtained using a MAP electrode (Linton Instruments, Harvard Apparatus). MAPs from the left ventricular endocardium were obtained using a custom-made MAP electrode that was made from two strands of 0.25 mm Teflon-coated silver wire (99.99% purity; Advent Research Materials, UK). The tips of the electrode were galvanically-chlorided to eliminate DC offset. The stimulating and recording electrodes were maintained at constant positions, with an inter-electrode distance of 3 mm. This allowed conduction velocities (CVs) to be

determined from the activation latencies. All recordings were performed using a baseline cycle length (BCL) of 125 ms (8 Hz) to exclude rate-dependent differences in action potential durations (APDs). MAPs were pre-amplified using a NL100AK head stage, amplified with a NL 104A amplifier and band pass filtered between 0.5 Hz and 1 kHz using a NL125/6 filter (Neurolog, Hertfordshire, UK) and then digitized (1401plus MKII, Cambridge Electronic Design, Cambridge, UK) at 5 kHz. They were then analysed using Spike2 software (Cambridge Electronic Design, UK). MAP waveforms that did not match the previous established stringent criteria for MAP signals were rejected (40). They must have stable baselines, fast upstrokes, with no inflections or negative spikes, and a rapid first phase of repolarization. 0% repolarization was measured at the peak of the MAP and 100% repolarization was measured at the point of return of the potential to baseline (40-42).

The following parameters were obtained from the experimental records: (1) Activation latency, defined as the time difference between the stimulus and the peak of the MAP; (2) CV, as the ratio of the inter-electrode distance to the activation latency. As the latter distance was kept constant, CVs were inversely proportional to the corresponding activation latencies; (3) APD_x, the time difference between the peak of the MAP and x = 30, 50, 70 and 90% repolarization; (4) ERP, defined as the longest S1S2 interval at which the S2 extrastimulus failed to initiate a ventricular signal during PES; (5) Excitation wavelength, λ, given by CV x ERP; (6) Critical intervals for re-excitation given by APD_{90} – ERP.

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Categorical data were compared with Fisher's exact test (one-tailed). Different experimental groups were

compared by one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. $P < 0.05$, 0.01 and 0.001 were denoted by *, ** and ***, respectively.

Results

Ventricular arrhythmogenicity and its relationship to action potential activation and recovery properties were examined before and after introduction of 0.05 mM heptanol in Langendorff-perfused mouse hearts. The right ventricular epicardium was electrically stimulated using either regular 8 Hz or S1S2 pacing (2, 3, 5, 7, 13). Monophasic action potential (MAP) recordings were obtained from the left ventricular epicardium. The stimulating and recording electrodes were maintained at a constant distance of 3 mm, which permitted conduction velocities (CVs) to be estimated from the respective activation latencies. Ventricular tachycardia (VT) was defined as a series of five or more action potentials with coupling intervals closer than the basic cycle length (BCL).

Heptanol at 0.05 mM exert ventricular pro-arrhythmic effects during S1S2 but not regular pacing

The initial experiments conducted during regular pacing demonstrated consistent ventricular activity in the absence of spontaneous arrhythmias in all of the 12 hearts studied whether before or after introduction of 0.05 mM heptanol or after removal of heptanol from the perfusate (Fig. 1a and 1b). The second set of experiments then applied a S1S2 pacing protocol, which imposed extrasystolic S2 stimuli following trains of regular S1 pacing stimuli. The S1S2 interval was initially at the BCL and subsequently reduced by 1 ms with each cycle until the S2 stimuli produced either arrhythmic activity or refractoriness. The latter indicating that the ERP was reached. None of the hearts studied demonstrated evidence of

inducible arrhythmias before application of the test agent (Fig. 2a). By contrast, it was possible to induce ventricular tachycardia (VT) after application of heptanol (Fig. 2b). The incidences of inducible VT before and after introduction of heptanol, and after its withdrawal from the perfusing solution are summarized in Fig. 2c, showing that heptanol exerted significant pro-arrhythmic effects, as the extrastimuli were able to induce VT in 6 out of 12 hearts (*asterisk*, Fisher's exact test, $P < 0.05$).

Pro-arrhythmic effects of heptanol were associated with decreased CVs in an absence of alterations in APDs or ERPs

Previous studies in mouse models have associated increased arrhythmogenicity with reduced CVs, prolonged or shortened APDs and reduce ERPs (2, 3, 5, 7, 13). These values were therefore obtained from the experimental recordings described above. Thus, heptanol increased activation latencies from 13.2 ± 0.6 to 19.4 ± 1.3 ms (Fig. 3a; ANOVA, $P < 0.001$) and reduced CVs from 0.23 ± 0.01 to 0.16 ± 0.01 ms (Fig. 3b, $P < 0.001$), without altering APD_{90} (38.0 ± 1.0 vs. 38.3 ± 1.8 ms; Fig. 3c), APD_{70} (16.8 ± 1.0 vs. 19.5 ± 0.9 ms; Fig. 3d), APD_{50} (9.2 ± 0.8 vs. 10.1 ± 0.6 ms; Fig. 3e), APD_{30} (4.8 ± 0.5 vs. 6.3 ± 0.6 ms; Fig. 3f) or ERPs (39.6 ± 1.9 vs. 40.6 ± 3.0 ms; Fig. 3g).

Pro-arrhythmic effects of heptanol were associated with decreased excitation wavelengths despite unaltered critical intervals

Decreases in excitation wavelengths (λ, CV x ERP) and increases in critical intervals for re-excitation (APD_{90} –ERP) have been associated with increased arrhythmogenicity (7, 43). Accordingly, these parameters were calculated for our hearts. Heptanol reduced λ from 9.1 ±

0.6 to 6.5 ± 0.6 mm (Fig. 4a, $P < 0.01$) without altering critical intervals (-1.1 ± 2.4 vs. -2.3 ± 1.8 ms; Fig. 4b).

Discussion

Sudden cardiac death (SCD) is a significant problem and is responsible for around 60,000 deaths in the U.K. (44), 200,000 deaths in the U.S. (45) and 4 to 5 million deaths globally (46) per year. It is likely to arise from the development of malignant ventricular arrhythmias, whose electrophysiological mechanisms remain incompletely understood. Mouse hearts have been used to study arrhythmogenesis as they are amenable to both genetic and pharmacological manipulation.

Propagation of the action potentials (APs) through the working myocardium depends on sodium channel activation followed by gap junction conduction. Gap junctions are hexameric proteins made of connexins mediate intercellular coupling by allowing passive electrotonic spread of ions and of larger molecules (47). Their resistances contribute to axial resistance and modulate conduction velocity (CV) (48, 49). Cx43 is the isoform found in ventricles, and the effects of Cx43 loss on ventricular conduction and arrhythmogenesis have been extensively studied in mouse models (27-35, 50, 51), but with significant disagreement between the studies (30). Thus, cardiac-restricted Cx43 inactivation followed by crossing with Cre recombinase produced mosaic mice, in which Cx43 was decreased by up to 95% when compared to wild-type (50). Other experiments found that heterozygous $Cx43^{+/-}$ mice showed 45 to 50% decrease in Cx43 expression. In these mice, CV was either unchanged (27-32) or decreased by 23 to 44% (33-35). These studies suggest different parameters, such as interstitial volume (52), width of the perinexus, intracellular calcium concentrations, perfusate composition and osmolarity (30), have additional effects on cardiac conduction.

Pharmacological methods have also been used to study the role of gap junctions in arrhythmogenesis. Thus, previous experiments reported that 2 mM heptanol exerted significant pro-arrhythmic effects by decreasing conduction velocities (CVs) without influencing action potential durations (APDs), but increased effective refractory periods (ERPs) (7). These changes led to reduced excitation wavelength (λ, CV x ERP) consistent with the increased likelihood of reentry. Heptanol is an agent that reversibly inhibits gap junctions at concentrations up to 1 mM and also sodium channels ≥ 2 mM (36, 38). It was therefore not possible to determine the relative contributions of gap junction uncoupling vs. reduced sodium channel function in the reduction of CV and the ventricular arrhythmogenesis observed. Furthermore, 2 mM heptanol produced not only CV slowing but also increased ERPs. The latter observation is consistent with its effects on sodium channel kinetics of producing a depolarizing shift of the activation curve and hyperpolarizing shift of the inactivation curve, which would reduce the sodium window current (38). Whilst increased ERP alone is expected to be anti-arrhythmic by increasing λ, regional increases in ERP could theoretically predispose to reentry by producing both refractory obstacles around which action potentials can circulate and areas of unidirectional conduction block (53).

Therefore, the present experiments were conducted to determine whether heptanol at a concentration that specifically gap junctions (0.05 mM) (36, 38) could produce pro-arrhythmic effects. Indeed, its application resulted in an increased incidence of inducible but not spontaneous arrhythmias, which was associated with increased activation latencies and decreased CVs, in an absence of alterations in APDs or ERPs. Together, these changes led to decreased excitation wavelength (λ) despite leaving critical intervals unaltered. These results complement previous findings that inhibition of both gap junctions and sodium channels at 2 mM heptanol resulted in a greater degree of CV slowing compared to the low concentration used here, and increased ERPs. Under these conditions, both spontaneous and provoked

ventricular tachycardia (VT) were observed. In the present study, gap junction inhibition alone using 0.05 mM heptanol did not elicit spontaneous VT during regular pacing.

As the aim of this study was to examine the effects of reducing gap junction coupling, it was therefore appropriate to use the MAP method. The latter has been extensively used to study cardiac electrophysiology in animal systems (9, 54, 55). For future experiments, measurement of magnetic signals has been useful for characterizing cardiac structural abnormalities (56-58) and recently, functional mapping could be achieved using magnetocardiography in mouse models, and its use in assess abnormal cardiac electrophysiology in mice therefore warrant future investigation (59).

In conclusion, this paper demonstrated that gap junction inhibition by heptanol alone was sufficient to reduce both CV without affecting APD or ERP, and the consequent decrease in λ was likely responsible for the arrhythmogenesis observed.

Figure legends

Figure 1. Stable monophasic action potentials (MAPs) obtained during regular 8 Hz pacing before (a) and after introduction of 0.05 mM heptanol (b).

Figure 2. Refractory outcomes observed before introduction of the test agent (a) and induced ventricular tachycardia after introduction of 0.05 mM heptanol (b) during S1S2 pacing.

Figure 3. Heptanol (0.05 mM) increased activation latencies (a) and reduced conduction velocities (CVs) (b) without affecting action potential durations at 90% (c), 70% (d), 50% (e) or 30% (f) repolarization (APD_x), or effective refractory periods (ERPs) (g).

Figure 4. Heptanol (0.05 mM) reduced excitation wavelengths (λ, CV x ERP) (a) without altering critical intervals for re-excitation (CI, APD_{90} – ERP) (b).

Acknowledgements

GT was awarded a BBSRC Doctoral Training Award at the University of Cambridge.

References

1. Tse G: Both transmural dispersion of repolarization and transmural dispersion of refractoriness are poor predictors of arrhythmogenicity: a role for the index of Cardiac Electrophysiological Balance (QT/QRS)? J Geriatr Cardiol 2016.
2. Tse G, Tse V and Yeo JM: Ventricular anti‑arrhythmic effects of heptanol in hypokalaemic, Langendorff‑perfused mouse hearts. Biomed Rep 4: 313-324, 2016.
3. Tse G, Tse V, Yeo JM and Sun B: Atrial anti-arrhythmic effects of heptanol in Langendorff-perfused mouse hearts. PLoS One 11: e0148858, 2016.
4. Tse G, Wong STT, V., Lee YT, Lin HY and Yeo JM: Cardiac dynamics: alternans and arrhythmogenesis. J Arrhythm 2016.
5. Tse G, Wong ST, Tse V and Yeo JM: Restitution analysis of alternans using dynamic pacing and its comparison with S1S2 restitution in heptanol-treated, hypokalaemic Langendorff-perfused mouse hearts. Biomed Rep 2016.
6. Tse G and Yeo JM: Conduction abnormalities and ventricular arrhythmogenesis: The roles of sodium channels and gap junctions. Int J Cardiol Heart Vasc 9: 75-82, 2015.
7. Tse G, Hothi SS, Grace AA and Huang CL: Ventricular arrhythmogenesis following slowed conduction in heptanol-treated, Langendorff-perfused mouse hearts. J Physiol Sci 62: 79-92, 2012.
8. Tse G: Mechanisms of Cardiac Arrhythmias. J Arrhythm 32: 75-81, 2015.
9. Tse G, Wong ST, Tse V and Yeo JM: Monophasic action potential recordings: which is the recording electrode? J Basic Clin Physiol Pharmacol 2016.
10. Tse G, Lai TH, Yeo JM, Tse V and Wong SH: Mechanisms of electrical activation and conduction in the gastrointestinal system: lessons from cardiac electrophysiology. Front Physiol 2016.
11. Tse G, Wong ST, Tse V and Yeo JM: Depolarization vs. repolarization: what is the mechanism of ventricular arrhythmogenesis underlying sodium channel haploinsufficiency in mouse hearts? Acta Physiol (Oxf) 2016.
12. Chen Z, Sun B, Tse G, Jiang J and Xu W: Reversibility of both sinus node dysfunction and reduced HCN4 mRNA expression level in an atrial tachycardia pacing model of tachycardia-bradycardia syndrome in rabbit hearts. Int J Clin Exp Pathol 2016.
13. Tse G, Sun B, Wong ST, Tse V and Yeo JM: Ventricular anti-arrhythmic effects of hypercalcaemia treatment in hyperkalaemic, Langendorff-perfused mouse hearts. Biomed Rep 2016.
14. Tse G, Lai ET, Tse V and Yeo JM: Molecular and electrophysiological mechanisms underlying cardiac arrhythmogenesis in diabetes mellitus. J Diabetes Res 2016.
15. Tse G, Lai ET, Yeo JM and Yan BP: Electrophysiological mechanisms of Bayés syndrome: insights from clinical and mouse studies. Front Physiol 2016.
16. Tse G, Wong ST, Tse V and Yeo JM: Determination of action potential wavelength restitution in Scn5a+/− mouse hearts modelling human Brugada syndrome. J Physiol 2016.

17. Osadchii OE: Flecainide-induced proarrhythmia is attributed to abnormal changes in repolarization and refractoriness in perfused guinea-pig heart. J Cardiovasc Pharmacol 60: 456-466, 2012.
18. Osadchii OE: Quinidine elicits proarrhythmic changes in ventricular repolarization and refractoriness in guinea-pig. Can J Physiol Pharmacol 91: 306-315, 2013.
19. Wilde AA, Postema PG, Di Diego JM, *et al*: The pathophysiological mechanism underlying Brugada syndrome: depolarization versus repolarization. J Mol Cell Cardiol 49: 543-553, 2010.
20. Osadchii OE: Impact of hypokalemia on electromechanical window, excitation wavelength and repolarization gradients in guinea-pig and rabbit hearts. PLoS One 9: e105599, 2014.
21. Osadchii OE: Impaired epicardial activation-repolarization coupling contributes to the proarrhythmic effects of hypokalaemia and dofetilide in guinea pig ventricles. Acta Physiol (Oxf) 211: 48-60, 2014.
22. Hsieh YC, Lin JC, Hung CY, *et al*: Gap junction modifier rotigaptide decreases the susceptibility to ventricular arrhythmia by enhancing conduction velocity and suppressing discordant alternans during therapeutic hypothermia in isolated rabbit hearts. Heart Rhythm 13: 251-261, 2016.
23. Hsieh YC, Lin SF, Huang JL, *et al*: Moderate Hypothermia (33C) Decreases the Susceptibility to Pacing-Induced Ventricular Fibrillation Compared with Severe Hypothermia (30C) by Attenuating Spatially Discordant Alternans in Isolated Rabbit Hearts. Acta Cardiol Sin 30: 455-465, 2014.
24. Hsieh YC, Lin SF, Lin TC, Ting CT and Wu TJ: Therapeutic hypothermia (30 degrees C) enhances arrhythmogenic substrates, including spatially discordant alternans, and facilitates pacing-induced ventricular fibrillation in isolated rabbit hearts. Circ J 73: 2214-2222, 2009.
25. Choy L, Yeo JM, Tse V, Chan SP and Tse G: Cardiac disease and arrhythmogenesis: lessons from mouse studies. Int J Cardiol Heart Vasc 2016.
26. Tse G, Lai ET, Yeo JM and Yan BP: What is the arrhythmic substrate in viral myocarditis? Insights from clinical and animal studies. Am J Transl Res 2016.
27. Stein M, van Veen TA, Remme CA, *et al*: Combined reduction of intercellular coupling and membrane excitability differentially affects transverse and longitudinal cardiac conduction. Cardiovasc Res 83: 52-60, 2009.
28. Stein M, van Veen TA, Hauer RN, de Bakker JM and van Rijen HV: A 50% reduction of excitability but not of intercellular coupling affects conduction velocity restitution and activation delay in the mouse heart. PLoS One 6: e20310, 2011.
29. Morley GE, Vaidya D, Samie FH, Lo C, Delmar M and Jalife J: Characterization of conduction in the ventricles of normal and heterozygous Cx43 knockout mice using optical mapping. J Cardiovasc Electrophysiol 10: 1361-1375, 1999.
30. George SA, Sciuto KJ, Lin J, *et al*: Extracellular sodium and potassium levels modulate cardiac conduction in mice heterozygous null for the Connexin43 gene. Pflugers Arch 2015.
31. Vaidya D, Tamaddon HS, Lo CW, *et al*: Null mutation of connexin43 causes slow propagation of ventricular activation in the late stages of mouse embryonic development. Circ Res 88: 1196-1202, 2001.
32. van Rijen HV, Eckardt D, Degen J, *et al*: Slow conduction and enhanced anisotropy increase the propensity for ventricular tachyarrhythmias in adult mice with induced deletion of connexin43. Circulation 109: 1048-1055, 2004.

33. Guerrero PA, Schuessler RB, Davis LM, *et al*: Slow ventricular conduction in mice heterozygous for a connexin43 null mutation. The Journal of clinical investigation 99: 1991-1998, 1997.
34. Thomas SA, Schuessler RB, Berul CI, *et al*: Disparate effects of deficient expression of connexin43 on atrial and ventricular conduction: evidence for chamber-specific molecular determinants of conduction. Circulation 97: 686-691, 1998.
35. Eloff BC, Lerner DL, Yamada KA, Schuessler RB, Saffitz JE and Rosenbaum DS: High resolution optical mapping reveals conduction slowing in connexin43 deficient mice. Cardiovasc Res 51: 681-690, 2001.
36. Christ GJ, Spektor M, Brink PR and Barr L: Further evidence for the selective disruption of intercellular communication by heptanol. Am J Physiol 276: H1911-H1917, 1999.
37. Rudisuli A and Weingart R: Electrical properties of gap junction channels in guinea-pig ventricular cell pairs revealed by exposure to heptanol. Pflügers Arch 415: 12-21, 1989.
38. Nelson WL and Makielski JC: Block of sodium current by heptanol in voltage-clamped canine cardiac Purkinje cells. Circ Res 68: 977-983, 1991.
39. Balasubramaniam R, Grace AA, Saumarez RC, Vandenberg JI and Huang CL: Electrogram prolongation and nifedipine-suppressible ventricular arrhythmias in mice following targeted disruption of KCNE1. J Physiol 552: 535-546, 2003.
40. Knollmann BC, Katchman AN and Franz MR: Monophasic action potential recordings from intact mouse heart: validation, regional heterogeneity, and relation to refractoriness. J Cardiovasc Electrophysiol 12: 1286-1294, 2001.
41. Gussak I, Chaitman BR, Kopecky SL and Nerbonne JM: Rapid ventricular repolarization in rodents: electrocardiographic manifestations, molecular mechanisms, and clinical insights. J Electrocardiol 33: 159-170, 2000.
42. Fabritz L, Kirchhof P, Franz MR, *et al*: Prolonged action potential durations, increased dispersion of repolarization, and polymorphic ventricular tachycardia in a mouse model of proarrhythmia. Basic Res Cardiol 98: 25-32, 2003.
43. Wiener N and Rosenblueth A: The mathematical formulation of the problem of conduction of impulses in a network of connected excitable elements, specifically in cardiac muscle. Arch Inst Cardiol Mex 16: 205-265, 1946.
44. NICE: Implantable cardioverter defibrillators for arrhythmias. Review of technology appraisal 11. National Institute for Health and Clinical Excellence (NICE) 2007.
45. Adabag AS, Luepker RV, Roger VL and Gersh BJ: Sudden cardiac death: epidemiology and risk factors. Nat Rev Cardiol 7: 216-225, 2010.
46. Chugh SS, Reinier K, Teodorescu C, *et al*: Epidemiology of sudden cardiac death: clinical and research implications. Prog Cardiovasc Dis 51: 213-228, 2008.
47. Spray DC and Burt JM: Structure-activity relationships of the cardiac gap junction channel. Am J Physiol 258: C195-C205, 1990.
48. Dhillon PS, Gray R, Kojodjojo P, *et al*: Relationship between gap-junctional conductance and conduction velocity in mammalian myocardium. Circ Arrhythm Electrophysiol 6: 1208-1214, 2013.
49. Peters NS: Gap junctions: clarifying the complexities of connexins and conduction. Circ Res 99: 1156-1158, 2006.
50. Gutstein DE, Morley GE, Tamaddon H, *et al*: Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. Circ Res 88: 333-339, 2001.
51. Beauchamp P, Choby C, Desplantez T, *et al*: Electrical propagation in synthetic ventricular myocyte strands from germline connexin43 knockout mice. Circ Res 95: 170-178, 2004.

52. Veeraraghavan R, Salama ME and Poelzing S: Interstitial volume modulates the conduction velocity-gap junction relationship. Am J Physiol Heart Circ Physiol 302: H278-286, 2012.
53. Hondeghem LM: QTc prolongation as a surrogate for drug-induced arrhythmias: fact or fallacy? Acta Cardiol 66: 685-689, 2011.
54. Vigmond EJ: The electrophysiological basis of MAP recordings. Cardiovasc Res 68: 502-503, 2005.
55. Vigmond EJ and Leon LJ: Electrophysiological basis of mono-phasic action potential recordings. Med Biol Eng Comput 37: 359-365, 1999.
56. Tse G, Ali A, Alpendurada F, Prasad S, Raphael CE and Vassiliou V: Tuberculous Constrictive Pericarditis. Res Cardiovasc Med 4: e29614, 2015.
57. Tse G, Ali A, Prasad SK, Vassiliou V and Raphael CE: Atypical case of post-partum cardiomyopathy: an overlap syndrome with arrhythmogenic right ventricular cardiomyopathy? BJR|case reports 1: 20150182, 2015.
58. Vassiliou V, Chin C, Perperoglou A, *et al*: 93 Ejection Fraction by Cardiovascular Magnetic Resonance Predicts Adverse Outcomes Post Aortic Valve Replacement. Heart 100: A53-A54, 2014.
59. Ono Y and Ishiyama A: Non-invasive Cardiac Functional Mapping on Disease-model Mice - Development of High Spatial Resolution SQUID System for MCG on Mice. TEION KOGAKU (Journal of Cryogenics and Superconductivity Society of Japan) 40: 44-50, 2005.