The official meeting of the ESC Working Group on Cardiac Cellular Electrophysiology

www.escardio.org/ewgcce-meeting

Cardiac Cellular Electrophysiology
ESC Working Group

EUROPEAN SOCIETY OF CARDIOLOGY®

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Welcome

Dear Participant,

Welcome to Glasgow, a vibrant and cosmopolitan place with a wealth of cultural heritage to explore. You will spend three days discussing the latest on Cardiac Cellular Electrophysiology. We are grateful for your interest and attendance to this meeting. Much has been done to make this a unique occasion: an excellent scientific program, excellent speakers and the warm ambiance of Glasgow. Especially, we thank you for submitting your scientific contribution.

We have had great fun in organizing this year's Scientific Meeting of the European Working Group on Cardiac Cellular Electrophysiology.
It would not have been possible without the involvement of the Scientific Committee, the Nucleus members of the Working Group and the Working Groups Office team at the European Heart House.
Enjoy and learn!

The local organizers,

Godfrey Smith, Francis Burton, Peter Macfarlane, Niall Macquaide, Rachel Myles, Antony Workman, Christopher Loughrey

General Information

Venue
Kelvin Gallery - University of Glasgow , Gilbert Scott Building, G12 8QQGlasgow, United Kingdom

Registration Desk
Your badge and the Meeting documentation can be collected on the first meeting day, Saturday 2 September at the venue, from 8.00 am.
Our staff at the registration desk will assist you in case of any inquiry.

Language
The offi cial language of the Meeti ng is English.

Badge
Participants of the Meeting are requested to wear their badge at all times during the Meeting.

Oral Presentations
The oral sessions will be held in the Kelvin Gallery, in the Gilbert Scott Building at the Meeting venue. Presenters are kindly asked to provide their presentati ons on USB stick well before the start of the session in which they are scheduled.

Poster Presentations
Posters will be displayed at the ground floor of the Meeti ng venue.
Poster dimensions should not exceed 130 cm width x 90 cm height.
Posters should be mounted on the poster boards on arrival to the Meeting venue and will be displayed on Saturday and Sunday. Posters should be removed Sunday before the evening program (18.00 h).

Coff ee/Tea
Coff ee, tea and water will be available during the breaks.

Lunch
Lunch is included in the registration fee and will be offered Saturday and Sunday at 12.30 and on

General Assembly
We kindly invite all EWGCCE members to attend the General Assembly scheduled on Sunday 4 September from 17.00 h – 18.00h. Important information will come by then, and this is also the moment when the poster prizes will be notified and awarded.

Welcome Reception
Friday 2nd September, from 18.00, the welcome reception will be held in the Hunterian Museum (Gilbert Scott Building - University of Glasgow).

Meeting Dinner
The Meeting dinner will be served in the Hilton Glasgow Grosvenor , 1-9 Grosvenor Terrace, Glasgow. Walking distance from the University. The dinner is included in the registration fee.

WiFi
At the Meeting venue, free, open, WiFi will be available.

Scientific Programme

Saturday 3rd September

08:30-10:00- Session 1: Ventricular arrhythmia mechanisms:

Chairs: Peter MacFarlane, Paul Volders

Title: Hereditary repolarization disorders: ECG imaging of the clinical substrate and mathematical modeling of the molecular mechanism
Speaker: **Yoram Rudy** (St Louis, MO) 40+5mins

Oral presentations: 3 x (10+5mins)

- The different possible mechanisms of the perpertuation of Torsade de Pointes in the drug-induced Chronic AV Block Dog - **N. Vandersickel**, A.Dunnink, A. Bossu, V. Meyboom, M. van der Heyden J. Beekman, J.M.T. de Bakker, M.A.Vos, A.V. Panfilov (Ghent BE)
- The differential effects of hypothermia on cardiac conduction and excitability -Karen McGlynn, **Erik Sveberg Dietrichs**, Andrew Allan, Adam Connolly, Martin Bishop, Francis Burton, Torkjel Tveita, Godfrey L Smith (Glasgow UK)
- Variability in the kinetics of cardiac I_{Na} - **Michael Clerx**, Roel L.H.M.G. Spätjens, Sandrine R.M. Seyen, Pieter Collins, Ralf L.M. Peeters, Paul G.A. Volders (Maastricht NL)

10:00-10.30- Coffee Break

10:30-12:00- Session 2: Atrial arrhythmia mechanisms:

Chairs: Tony Workman, Dobromir Dobrev

Title: Translational assessment of cellular mechanisms of atrial fibrillation
Speaker: **Uli Schotten** (Maastricht, Netherlands) 40+5mins

Oral presentations: 3 x (10+5mins)

- A dynamic-clamp study of L-type Ca^{2+} current in rabbit and human atrial myocytes: the contribution of window I_{CaL} to early afterdepolarisations -Kettlewell S, Dempster J, Colman MA, Rankin AC, Myles RC, Smith GL, **Workman AJ** (Glasgow UK)
- Loss of myocardial nNOS begets atrial fibrillation by abolish the physiological right-left action potential duration gradient in human and mouse atrial myocytes -**Xing Liu**, Ricardo Carnicer, Alice Recalde, Alfonso Bueno-Orovio, Blanca Rodriguez, Barbara Casadei (Oxford UK)

PDE8 is a novel regulator of cAMP signaling in human atrial fibrillation - **C E Molina**, S Ghezelbash, E Jacquet, A Garnier, R Fischmeister, D Dobrev (Essen DE)

12:30 – 13:30: Lunch

14:00-15:30- posters (number 1 to number 43)

15:30-16:00- Coffee Break

16:00-17:30- Keynote CCW lecture: Denis Noble (Oxford, UK)
The golden trio of cardiac electrophysiology, Coraboeuf, Carmeliet & Weidmann, chasing thresholds and conductance changes in the early days of micro electrode recording.

19:00 - Conference dinner

At the Hilton Glasgow Grosvenor
1-9 Grosvenor Terrace, Glasgow

Sunday 4th September

08:30-10:00- Session 3: Emerging therapies to address myocardial disease

Chairs: Godfrey Smith, Matteo Mangoni
Title: iPS-cell derived engineered heart tissue improves left-ventricular function after myocardial injury.
Speaker: **Thomas Eschenhagen** (Hamburg Germany) 40+5min

Oral presentations: 3 x (10+5mins)

- Elevated Ventricular Wall Stress Disrupts Cardiomyocyte T-tubular Structure and Ca^{2+} Homeostasis - **Ruud M**, Frisk M, Espe EK, Aronsen M, Zhang L, Norseng PA, Sjaastad I, Sejersted O, Christensen G, Louch WE (Oslo NO)
- Measuring electrical conductivity of cardiac T-tubular systems - **M. Scardigli**, C. Crocini, C. Ferrantini, T. Gabbrielli, L. Silvestri, C. Tesi, E. Cerbai, C. Poggesi, F. S. Pavone, L. Sacconi (Sesto Fiorentino IT)
- Intramural structural discontinuities underlie right ventricular conduction abnormalities in the Scn5a haplo-insufficient mouse model - **Allen Kelly**, Simona Salerno, Adam Connelly, Martin Bishop, Ulrik Wisloff, Flavien Charpentier, Tomas Stolen, Godfrey Smith (Glasgow UK)

10:00-10.30- Coffee Break

10:30-12:00- Session 4: Excitation-contraction coupling in health and disease

Chairs: Chris Loughrey, Frank Heinzel

Title: Control of diastolic calcium.
Speaker: **David Eisner (**Manchester UK)

Oral presentations: 3 x (10+5mins)

- The efficacy of late sodium current blockers in hypertrophic cardiomyopathy is dependent on genotype: a study on transgenic mouse models with different mutations.
- **L. Santini**, R. Coppini, L. Mazzoni, C. Ferrantini, F. Gentile, JM. Pioner, L. Sartiani, C. Poggesi, A. Mugelli, E. Cerbai (Florence IT)
- Physical coupling between SERCA2 and PDE3A regulates SERCA2 activity in cardiomyocytes
- Jan Magnus Aronsen MD, **Jonas Skogestad** MD, Karina Hougen MD PhD, Marianne Lunde, Gustav Lothe, Per Kristian Lunde PhD, Jens Preben Morth PhD, Kjetil Taskén MD PhD, Cathrine Rein Carlson PhD, Ivar Sjaastad MD PhD (Oslo NO)

- Phosphodiesterase-5 inhibitor sildenafil suppresses calcium waves by reducing sarcoplasmic reticulum content - **D. Hutchings**, K. Dibb, D. Eisner, A. Trafford (Manchester UK)

12:15 – 13:15: Lunch

13:30-15:00- posters (number 44 to number 86)

15:30-17:00- Session 5: Novel pathways regulating cardiac electrophysiology

Chairs: Rachel Myles, Carol Ann Remme

Title: The role of sarcoplasmic reticulum calcium handling during alternans and fibrillation
Speaker: **Crystal Ripplinger** (Davis CA) 40+5min

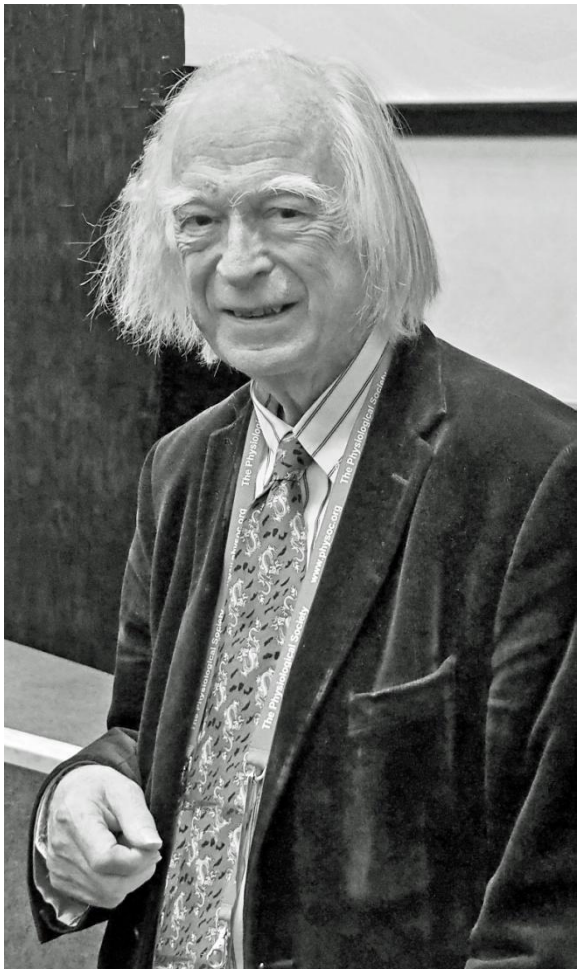
Oral presentations: 3 x (10+5mins)

- Optogenetic termination of atrial fibrillation in the mouse heart
- **Tobias Bruegmann**, Thomas Beiert, Jan Wilko Schrickel, Philipp Sasse (Bonn DE)
- Optical treatment of cardiac arrhythmias
- **Claudia Crocini**, Cecilia Ferrantini, Raffaele Coppini, Marina Scardigli, Leslie M. Loew, Godfrey Smith, Corrado Poggesi, Elisabetta Cerbai, Francesco S. Pavone, Leonardo Sacconi (Sesto Fiorentino IT)
- Optogenetic monitoring of endocardial calcium transients *in vivo* using a minimally invasive fiber optic approach. L. Menke, I. van Asten, L. van Stuijvenberg, T.A.B. van Veen, M.A. Vos, **T P. de Boer** (Utrecht NL)

17:00-18:00 - General Assembly

Carmeliet-Coraboeuf-Weidmann lecture

Denis Noble FRS



Biosketch: Denis Noble hardly needs any detailed introduction to this meeting since his work initiated, then stimulated, informed and underpinned that of so many in the field and is surely well known to you all. Denis is Emeritus Professor of Cardiovascular Physiology at the University of Oxford where he held the Burdon Sanderson Chair from 1984 to 2004. Extending from Hodgkin and Huxley's pioneering mathematical description of the propagated nerve action potential, he developed the first mathematical electrophysiological models of cardiac cells. This work began in 1960 (at University College London) using the discovery, made with his PhD supervisor Otto Hutter, of two of the main cardiac potassium ion channels. A core concept is that there is no 'primary oscillator' but that the heart's oscillatory pacemaking behaviour is an 'emergent property' of the complex electrophysiological and biophysical attributes of multicellular cardiac tissue. The project was later developed extensively with Dick Tsien, Dario DiFrancesco, Don Hilgemann and others, including Denis' late wife Susan, to provide the canonical models on which over a hundred cardiac cell models are based today.

Denis' insights as expressed in cardiac modelling found a wider platform. He was a co-founder of the 'Physiome Project', initially presented as a report from the Commission on Bioengineering in Physiology to the International Union of Physiological Sciences (IUPS) Council at the Glasgow Congress in 1993: its declared intent is to provide a "quantitative description of physiological dynamics and functional behaviour of the intact organism". At the Glasgow Congress, a seminal book given to attendees *The Logic of Life* (co-edited with Richard Boyd) revealed the potential breadth of the conceptual framework underpinning to the physiome project. In the preface, a quote from Nobel Laureate Sir James Black captured the mood with Sir James seeing the future of his science as "the progressive triumph of physiology over molecular biology".

In 2009, Denis was elected President of IUPS at its Kyoto Congress and then for a second term (in Birmingham, UK) in 2013. He delivered the opening plenary lecture at the Birmingham Congress, published as an article in *Experimental Physiology* (**98**, 1235-1243, 2013). Here his recent thinking towards a new understanding of evolutionary biology was expounded. This article, and other works in a similar vein, led to the special issue of the *Journal of Physiology* (**592**, June 2014) '*The*

integration of evolutionary biology with physiological science'. This issue was provocatively sub-titled in the editorial '*Evolution evolves: physiology returns to centre stage*'. Some 35 eminent authors contributed to the 19 papers published there.

Denis wrote what is perhaps the first popular book on Systems Biology, *The Music of Life*. It has rightly enjoyed widespread critical acclaim. Over the last decade and more, his lectures and presentations have principally concerned the implications of physiological knowledge for evolutionary biology and *vice versa*. Denis has established himself as one of the leading thinkers in modern bioscience. (That rather fuzzy notion of the 'public philosopher' perhaps fits him well.) His deep scholarship ranges over the central ideas of biology and of the philosophy of science and is truly that of a polymath. As a physiologist and a leading proponent of the notion of 'emergence', he will relish that he continues a strand of philosophical thought whose name was coined by a founder member of The Physiological Society, George Henry Lewes (1817-1878).
Denis has published more than 500 papers as well as 11 books. His latest opus *Dance to the Tune of Life; Biological Relativity* will soon be published (by Cambridge University Press).

David Miller July 2016

Edward Carmeliet

Edouard Coraboeuf

SilvioWeidmann

The CCW lecture has been established to celebrate the contributions that Edward Carmeliet, Edouard Coraboeuf and Silvio Weidmann have made to cardiac cellular electrophysiology. It also recognizes their roles in establishing the Working Group in Cardiac Cellular Electrophysiology, which later became part of the European Society of Cardiology.
With the kind permission of Edward Carmeliet and the families of Edouard Coraboeuf and Silvio Weidmann, the lectureship is awarded annually to an outstanding European Cardiac Cellular Electrophysiologist. The story begins with Silvio Weidmann (1921-2005). After studying medicine at the University of Bern, in 1948 he went to the University of Cambridge to work with Alan Hodgkin and Andrew Huxley who were at that time well on their way to elucidating the properties of the nerve action potential. In Cambridge, Silvio was joined by Edouard Coraboeuf (1926- 1998). Together, in 1949, they published the first intracellular recording of a cardiac action potential. Edward Carmeliet also worked with Silvio Weidmann, in his case in Bern, where he carried out his PhD with pioneering studies on the potassium and chloride permeability of the heart. All three continued to make outstanding contributions to cardiac electrophysiology. Silvio Weidmann demonstrated the low conductance of the plateau of the action potential and the voltage dependence of the sodium channel as well as its sensitivity to local anesthetics. He also

demonstrated the diffusion of potassium between cells. Edouard Coraboeuf went on to identify early afterdepolarizations, which lead to torsades-de-pointes arrhythmias. He subsequently pioneered cellular studies on the human heart as well as characterizing the maintained component of the sodium current and its contribution to the plateau of the action potential. As mentioned above, Edouard and Edward had both worked with Silvio. Subsequently the two collaborated on work characterizing the chloride current. Edward Carmeliet (1930-) also pioneered studies of the control of the action potential duration; in particular the effects of heart rate and metabolism. He published seminal papers on virtually every cardiac potassium channel and on the mechanisms of action of antiarrhythmic agents. He also demonstrated the interaction of ionic gradients with channels and transporters.

As well as their own scientific contributions, all three have established their own schools of research as represented by countless successful careers of younger scientists worldwide.

Our Working Group owes much to this trio. Edward Carmeliet organized the first meeting in Leuven in 1977. The next year Edouard Coraboeuf organized a meeting in Orsay and, in 1980, the Working Group met in Bern at the invitation of Silvio Weidmann.

Past CCW Lecturers:
2012: Ursula Ravens (36th EGWCCE Meeting, Nantes, FR)
2013 David Eisner (37th EGWCCE Meeting, Athens, GR)
2014: András Varró (38th EWGCCE Meeting, Maastricht, NL)
2015: Barbara Casadei (39th EWGCCE Meeting, Milan, IT)

The CCW lecture is supported by CAIRN Research

Posters

Saturday 3rd September

Poster presenters should be available at their poster from 14:00 – 15:30

Poster number 1 to poster number 43

1. Short QT in a murine model of metabolic dysfunction
Shiraz Ahmad, Haseeb Valli, Samantha Salvage, Andrew Grace, Kamalan Jeevaratnam, Christopher Huang.
Centre of affiliation: University of Cambridge. London, UK.

2. Biphasic time course of response to activation of sympathetic nerves in isolated rabbit ventricular myocardium
Stephanie Anderson, Francis Burton, Godfrey Smith, Rachel Myles.
Centre of affiliation: British Heart Foundation funded PhD student. Glasgow, UK.

3. Risk assessment models for myocardial infarction
Galya Atanasova, PhD.
Centre of affiliation: University Hospital, Department of Internal Medicine. Pleven, Bulgaria.

4. Rad GTPase as a new potential actor in Brugada syndrome
Nadjet Belbachir, Vincent Portero, Eva LePogam, Nathalie Gaborit, Solena Le Scouarnec, Isabelle Baró, Christophe Guilluy, Celine Marionneau, Vincent Probst, Jean-Jacques Schott, Richard Redon, Flavien Charpentier.
Centre of affiliation: l'institut du thorax. Nantes, France.

5. Modifier genes in the LQT1 syndrome: mechanistic analysis of NOS1AP polymorphism
Joyce Bernardi, Carlotta Ronchi, Eleonora Torre, Marcella Rocchetti, Antonio Zaza.
Centre of affiliation: University of Milano-Bicocca. Milan, Italy.

6. The arrhythmogenic electrophysiological and structural substrates of the RVOT: insights from large animal models and human hearts
D. Benoist, V. Dubes, M. Constantin, S. Charron, S. H. Gilbert, C. N. W. Belterman, M. Haissaguerre, E. White, R. Coronel, O. Bernus.
Centre of affiliation: Electrophysiology and Heart Modeling Institute LIRYC - University of Bordeaux. Bordeaux, France.

7. Effects of exercise training on myocardial and skeletal muscle function in a post-myocardial infarction heart failure rat model
Aline Bezerra Gurgel, Michael Dunne, Godfrey Smith, Ole Kemi.
Centre of affiliation: University of Glasgow. UK.

8. Rotors in AF and impact of ablation

Caroline Cros, Remi Chayvel, Richard Walton, Caroline Auclerc-Pascarel, Valentin Meillet, Remi Dubois, Olivier Bernus, Pierre Jais, Fabien Brette.

Centre of affiliation: IHU LIRYC. Bordeaux, France.

9. A simple method to extract movement information from video images of contracting heart cells

Francis Burton.

Centre of affiliation: Institute of Cardiovascular and Medical Sciences, University of Glasgow. UK.

10. Are transverse tubules restored in the atria following recovery from heart failure?

J.L. Caldwell, J D Clarke, D.A. Eisner, A.W. Trafford, K.M. Dibb.

Centre of affiliation: University of Manchester.UK.

11. Absence of Nav1.8-based (late) sodium current in rabbit cardiomyocytes and human iPSC derived cardiomyocytes

Simona Casini, Isabella Mengarelli, Cees A. Schumacher, Marieke W. Veldkamp, Arie O. Verkerk, Carol Ann Remme.

Centre of affiliation: Experimental Cardiology. Amsterdam, The Netherlands.

12. Alpha1-adrenergic regulation of hERG Current is mediated by PKC dependent channel phosphorylation

Janire Urrutia, Aintzane Alday, Layse Malagueta-Vieira, Monica Gallego, Oscar Casis.

Centre of affiliation: Universidad del País Vasco UPV/EHU. Vitoria-Gasteiz, Spain.

13. The threshold behavior of the cardiac sodium current is potentiated by ephaptic effects: insights from a high resolution mathematical model of a narrow intercellular cleft

Echrak Hichri, Hugues Abriel, Jan P. Kucera.

Centre of affiliation: University of Bern. Switzerland.

14. Massively parallel all-optical cardiac electrophysiology

Emilia Entcheva.

Centre of affiliation: George Washington University. Washington, USA.

15. Arrhythmias and electrophysiological abnormalities in a mouse model of hypertrophic cardiomyopathy

Frederik Flenner, Felix W. Friedrich, Katrin Gurr, Klaus-Dieter Soehren, Thomas Eschenhagen, Torsten Christ, Lucie Carrier.

Centre of affiliation: Department of Experimental Pharmacology, UKE Hamburg. Germany.

16. Sodium channel expression, distribution and (dys)function in atrial myocytes: relevance for arrhythmogenesis

G.A. Marchal, S.Z. Ibrahim, C. Schumacher, M.W. Veldkamp and C.A. Remme.

Centre of affiliation: Academic Medical Centre. Amsterdam, The Netherlands.

17. Enhanced expression and PKCdelta-mediated hyperphosphorylation underlie the proarrythmic increase in sodium-calcium-exchanger activity in patients with chronic atrial fibrillation

Ghezelbash S, Molina CE, Badimon L, Kamler M, Heijman J, Dobrev D.

Centre of affiliation: Institute of Pharmacology, University Duisburg Duisburg-Essen. Germany.

18. Circadian rhythm in QT interval is preserved in Cardiomyocyte specific Bmal1 knockout mice despite changed J wave morphology

Lisa A Gottlieb, Morten B Thomsen.

Centre of affiliation: Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen. Denmark.

19. The effects of TBQ on cardiac intracellular ATP

Natasha Hadgraft, Mustafa Naeem, Gina Galli, Louise Miller, David Greensmith.

Centre of affiliation: University of Salford. UK.

20. Long-term stimulation of iPS-derived cardiomyocytes using optogenetic techniques to promote phenotypic changes in E-C coupling

Craig Hamilton, Niall MacQuaide, Victor Zamora, Godfrey Smith.

Centre of affiliation: University of Glasgow. UK.

22. Inward rectifier ion currents in human induced pluripotent stem cell-derived cardiomyocytes

András Horváth, Ahmet Uzun, Ingra Mannhardt, Kaja Breckwoldt, Christiane Neuber, Alexandra Löser, Arne Hansen, András Varró, Thomas Eschenhagen, Torsten Christ.

Centre of affiliation: UKE Hamburg, Department of Experimental Pharmacology and Toxicology. Hamburg, Germany.

23. Effects of Omecamtiv mecarbil on cellular electrophysiology and contractile properties of canine left ventricular myocytes

Balázs Horváth, Norbert Szentandrássy, Krisztina Váczi, Kornél Kistamás, Tamás Bányász, János Magyar, Péter P. Nánási.

Centre of affiliation: Department of Physiology, Faculty of Medicine, University of Debrecen. Hungary.

24. Antiarrhythmic treatment of ventricular fibrillation due to acute myocardial infarction in rats

Laura Amalie Hundahl, Lasse Skibsbye, Thomas Jespersen.

Centre of affiliation: Cardiac Physiology Group, Department of Biomedical Sciences. Copenhagen, Denmark.

25. Regulation of basal and norepinephrine-increased Ca^{2+}-currents by PDEs in human heart: differences between stem cell-derived and mature cardiomyocytes

Ismaili D, Mannhardt I, Hansen A, Eschenhagen T, Christ T.

Centre of affiliation: University Medical Center Hamburg-Eppendorf. Hamburg, Germany.

26. Allosteric modulator LUF7244 uncouples dofetilide-mediated rescue of aberrant hERG trafficking from dofetilide-induced hERG blockade

Y. Ji, M.J.C. Houtman, F. Romunde, D. Fransen, A.P. IJzerman, L.H. Heitman, M.A. Vos, M.A.G. van der Heyden.

Centre of affiliation: University Medical Center Utrecht. UTRECHT. The Netherlands.

27. Receptor-species dependent desensitization controls I_{Ks} as a downstream effector of Gq protein-coupled receptors

Marie-Cécile Kienitz, Dilyana Vladimirova, Christian Müller, Lutz Pott and Andreas Rinne.
Centre of affiliation: Department of Cellular Physiology, Ruhr-University. Bochum, Germany.

28. Dependence of diastolic calcium levels on frequency and extracellular calcium concentration

Kornel Kistamas, Luigi Venetucci, Andrew Trafford, David Eisner.

Centre of affiliation: Institute of Cardiovascular Sciences, Faculty of Biology, Medicine and Health, University of Manchester. Manchester, UK.

29. Human-induced pluripotent stem cell-derived cardiomyocytes: phenotypic and functional variability

Jussi Koivumaeki, Nikolay Naumenko, Tomi Tuomainen, Jouni Takalo, Pasi Tavi.

Centre of affiliation: University of Eastern Finland. Kuopio, Finland.

30. Dispersion of ryanodine receptor clusters disrupts calcium sparks in failing cardiomyocytes

Kolstad.T, MacQuaide.N, Edwards.A.G, Aronsen.J.M, Frisk.M, Sjaastad.I, Sejersted.O.M, Louch.W.E.

Centre of affiliation: Oslo University Hospital Ullevål and Institute for Experimental Medical Research. Oslo, Norway.

31. Optogenetic modulation of cardiomyocyte excitability

Ramona Kopton, Eva Rog-Zielinska, Urszula Siedlecka, Jonas Wietek, Peter Hegemann, Peter Kohl, Franziska Schneider.

Centre of affiliation: Institute for Experimental Cardiovascular Medicine, University Heart Centre Freiburg - Bad Krozingen, Medical Center - University of Freiburg, Germany. Freiburg, Germany.

32. Two pore mutations convert the depolarized-activated Shaker Kv channel into a hyperpolarized-conducting cation selective channel

Alain J. Labro, Evelyn Martinez-Morales, Dirk J. Snyders.

Centre of affiliation: University of Antwerp. Antwerp, Belgium.

33. Screening of adult rabbit cardiomyocytes action potential characteristics using FluoVolt and di-4-ANEPPS indicators

Quentin Lachaud, Niall MacQuaide, Francis Burton, Godfrey Smith.

Centre of affiliation: University of Glasgow. UK.

34. The Rho kinase inhibitor, fasudil, ameliorates diabetes-induced cardiac dysfunction via improving calcium removal modulation and actin remodeling

Lai Dongwu, Gao jing, Bi xukun, He Hong, Shi xiao lu, Yang ying, Ye yang, Fu guosheng.
Centre of affiliation: division of cardiology, sir run run shaw hospital, Zhejiang University. Hangzhou, China.

35. The transcription factor NFIX: a novel modulator of cardiac rhythm in the adult heart

S. Landi, G. Campostrini, V. Fontana, L. Carnevali, G. Rossi, G. Messina, A. Bucchi, M. Baruscotti, D. DiFrancesc, A. Barbuti.

Centre of affiliation: Department of Biosciences, University of Milan. Italy.

36. Ultrasound-guided venous access for pacemakers and defibrillators. Randomized trial.

Mattia Liccardo Pasquale Nocerino Antonella Borrino Cristina Carbone Gaia Salzano.
Centre of affiliation: Ospedale Santa Maria delle Grazie, Pozzuoli, Napoli. Italy.

37. Sarcolemmal structure and function reverts to an immature phenotype in failing cardiomyocytes Lipsett DB, Frisk M, Aronsen JM, Sjaastad I, Sejersted OM, Christensen G, Louch, WE.

Centre of affiliation: Institute for Experimental Medical Research, University of Oslo & Oslo University Hospital. Oslo, Norway.

38. Mapping the *in vitro* interactome of cardiac NCX1

T. Lubelwana Hafver, G.A. de Souza, P. Wanichawan, M. Lunde, M. Martinsen, O.M. Sejersted, C.R. Carlson.
Centre of affiliation: Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo. Norway.

39. Altered RyR structure and function in static culture

Connor Blair, Bracy Fertig, Godfrey Smith, Niall MacQuaide.
Centre of affiliation: University of Glasgow. UK.

40. Voltage dependence mechanism of the cardiac potassium channel hERG: a ligand/ receptor model

Olfat A. Malak, Zeineb Es-Salah-Lamoureux, Gildas Loussouarn.
NB: These authors contributed equally to this work.
Centre of affiliation: Inserm, UMR 1087, Institut du thorax, Université de Nantes. France.

41. Epac2 inhibition reduces arrhythmogenic Ca^{2+} wave frequency in cardiomyocytes from mice with catecholaminergic polymorphic ventricular tachycardia type 1

Sadredini M, Manotheepan R, Danielsen TK, Lehnart SL, Sjaastad I, Stokke MK.
Centre of affiliation: Institute for Experimental medical research. Oslo, Norway.

42. The coxsackievirus and adenovirus receptor regulates calcium homeostasis in the developing heart

Claudia Matthäus, René Jüttner, Fritz G. Rathjen.
Centre of affiliation: Max-Delbrück-Center for Molecular Medicine. Berlin, Germany.

43. L-type Cav1.3 and T-type Cav3.1 calcium channels in cardiac pacemaker activity

Matthias Baudot, Matteo Mangoni, Pietro Mesirca, Isabelle Bidaud, Antony Chung You Chong, Leila Talssi.
Centre of affiliation: Institut de génomique fonctionnelle. Montpellier, France.

Sunday 4th September:

Poster presenters should be available at their poster from 13:30-15:00

Poster number 44 to poster number 86

44. Importance of Cav1.3-mediated Ca^{2+} current in relation to I_f and Na^+/Ca^{2+} exchanger during the diastolic depolarization of mouse pacemaker cells
Mesirca P., Bidaud I., Striessnig J., Mangoni M.E.
Centre of affiliation: Institute for Functional Genomics. Montpellier, France.

45. Ageing alters calcium spark characteristics in the mouse sinus node
Louise Miller, Derek S. Steele, George Hart, Mark R. Boyett, Halina Dobrzynski.
Centre of affiliation: University of Manchester. UK.

46. Priming for action: interventions that promote I_{Ks}-channel opening restore defective cAMP-dependent upregulation in Long-QT1 syndrome
Cristina Moreno Vadillo, Roel L.H.M.G. Spätjens, Sandrine R.M. Seyen, Gabriele Menini, Paul G.A. Volders.
Centre of affiliation: CARIM institute, Maastricht University. The Netherlands.

47. Computer models of human atrial myocytes and whole atria facilitate experimental investigations into nNOS-mediated mechanisms for atrial fibrillation
Anna Muszkiewicz, Xing Liu, Alfonso Bueno-Orovio, Jose F. Rodriguez, Barbara Casadei, Blanca Rodriguez.
Centre of affiliation: University of Oxford. UK.

48. Calcium handling in pigs with progressive septic shock
Nalos L, Jarkovská D, Horák J, Beneš J, Martínková V, Švíglerová J, Matějovič M, Štengl M.
Centre of affiliation: Charles University in Prague, Medical Faculty in Pilsen. Plzeň.

49. Role of Iroquois transcription factors in cardiac arrhythmic diseases using induced pluripotent stem cells
Gaborit Nathalie, N. Gaborit, Z. Al Sayed, N. Jacob, D. Harkous, V. Forest, A. Girardeau, G. Lamirault, P. Lemarchand.
Centre of affiliation: L'institut du thorax, INSERM U1087. Nantes, France.

50. Einstein's equation and the role of electrons in cardiac electrophysiology
Mark I.M. Noble.
Centre of affiliation: University of Aberdeen. Scotland.

51. The rate-dependent effects of I_f blockade are influenced by exercise training in man
Charles M Pearman, Jessica Coulson, Laura C Smith, Moinuddin Choudhury, Emeka Oguguo, Mark Boyett, Gwilym M Morris.
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86. A possible mechanism underlying the weak phenotype in Long QT syndrome type 5

Balazs Ordog, Teodora Hartai, Szilvia Deri, Laszlo Virag, Norbert Jost, Isvtan Baczko, Andras Varro
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Best of Science selection @ 40th EWGCCE Meeting

Optogenetic termination of atrial fibrillation in the mouse heart

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Atrial fibrillation (AF) is the most common arrhythmia of the atrium with an increased risk of thrombus formation and stroke. Restoration of sinus rhythm is still the main goal in the initial treatment of patients with AF, especially if symptomatic. Because of the low efficacy of pharmaceutic termination of AF, electrical cardioversion using high amplitude electrical current is often the only option. Because these electrical shocks are painful for the patients, this has to be performed during general anesthesia and implantable AF cardioverters are not accepted by patients.

In clear contrast to electrical current, optogenetic methods enable cell-type selective stimulation of cells expressing light-sensitive proteins with light. Optogenetic stimulation of the heart has been demonstrated before in transgenic embryonic zebrafish and adult mice expressing the light-gated non-selective cation channel Channelrhodopsin2 (ChR2). Importantly, constant illumination leads to constant depolarization and refractoriness of ChR2-expressing cardiomyocytes *in vitro* suggesting that optogenetics could be used for the termination of AF.

To proof this principle we have performed experiments with Langendorff perfused hearts from double transgenic mice expressing ChR2 and the AF-promoting loss-of-function A96S mutation in Connexin40. ECG and atrial electrogramms were recorded by bipolar surface electrodes and a 2-French octapolar mouse electrophysiological catheter (CibaMouse, NuMED Inc).
Vulnerability for AF induction was enhanced by perfusion with the atrial K_{ATP}-channel activator Diazoxide (300 µM) in low K^+ (2mM) Tyrode solution. Atrial arrhythmia were induced by atrial epicardial electrical burst (5 s long, 30-100 Hz, 2 ms pulses, 2-10 mA) stimulation protocols.
This procedure induced long lasting episodes of AF or atrial flutter that could be optogenetically terminated by epicardial illumination of the atria. We found that 1 s long light pulses focused on both atria (100 mm^2) with 0.4 mW/mm^2 terminated the arrhythmia in all tested mice (n=7) with an average efficacy of each illumination protocol of 91.4 ± 8.6% (s.e.m.).
In preliminary experiments, we investigated different illumination parameters and found decreased efficacies with reduced light intensity, smaller size of the illuminated area and shorter illuminations. Importantly, optogenetic termination of AF was also effective by 1 sec long illuminations through a small light guide (Ø 400 µm core, 0.48 N.A., 70 mW/mm^2 at the tip) placed epicardially on the right atrium demonstrating how illumination could be performed in an implantable device.
In summary we provide the first evidence for optogenetic termination of atrial tachyarrhythmia. This report could lay out the foundation for the development of implantable devices for pain-free termination of atrial flutter and AF.

PDE8 is a novel regulator of cAMP signaling in human atrial fibrillation

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Purpose: Atrial fibrillation (AF) is associated with reduced L-type Ca^{2+} current ($I_{Ca,L}$) and altered cAMP-dependent signaling. Cyclic nucleotide phosphodiesterases (PDEs) degrade cAMP and regulate cAMP-mediated PKA-dependent phosphorylation of various proteins, including $I_{Ca,L}$ channel subunits. PDE1-4 are the main PDE isozymes hydrolyzing cAMP in heart, but recent studies demonstrate the existence of a novel isoform PDE8 in ventricle. Here we assess the expression, localization and function of PDE8 in human atria of patients with sinus rhythm (SR), paroxysmal AF (pAF) and longstanding persistent (chronic) AF (cAF).

Methods: mRNA (RT-qPCR) and protein (Western blot) levels of PDE8A and PDE8B isoforms were assessed in right atria of SR, pAF and cAF patients. Localization of PDE8A and PDE8B in human atrial cardiomyocytes was determined by immunofluorescence. Protein-protein interaction between $I_{Ca,L}$ α1C channel subunit and PDE8B was studied by co-immunoprecipitation in the three rhythm groups. Multicellular action potentials (APs) were recorded at 1 Hz in right atrial trabeculae from patients in SR, pAF and cAF.

Results: PDE8A mRNA is present in human atrium and increases significantly in pAF (ratio SR=0.93±0.04 n=16 *vs* pAF=1.13±0.04 n=8 and cAF =1.08±0.05 n=8, $p<0.01$, ANOVA). By contrast, in samples from cAF patients only PDE8B mRNA was increased (ratio SR=0.94±0.07 n=16 *vs* pAF=1.04±0.1 n=8 and cAF =1.28±0.13 n=8, $p<0.05$, ANOVA). Accordingly, GAPDH-normalized protein expression levels of PDE8A were 2-fold higher in pAF, but unaltered in cAF patients while PDE8B protein abundance was increased by ~77% in cAF only ($p<0.05$). Immunostaining confirmed the presence of PDE8A and PDE8B in human atrial cardiomyocytes, with PDE8A being localized in the cytosol, and PDE8B preferentially localized at the plasma membrane. Immunoprecipitation of $I_{Ca,L}$ α1C subunit resulted in strongly enhanced co-immunoprecipitation of PDE8B in cAF but not pAF (PDE8B/α1C ratio, SR=0.31±0.28 n=5, pAF =0.14±0.08 n=4, cAF mean=4.97±2.21 n=5, $p<0.05$, ANOVA), identifying PDE8B as part of the $I_{Ca,L}$ channel complex and pointing to potential contribution of PDE8B to reduced $I_{Ca,L}$ in cAF. Finally, preliminary results indicate that the selective PDE8 inhibitor PF-04957325 (1 µM) increased the plateau potential to more positive values and slightly prolonged the AP duration at 50% of repolarization, which is consistent with a stimulation of $I_{Ca,L}$.

Conclusions: Our results show for the first time that PDE8A and B are expressed in human atrium. PDE8B localizes at the plasma membrane of human atrial cardiomyocytes, and upregulates and accumulates in the $I_{Ca,L}$ channel complex of cAF patients, likely contributing to the reduction of $I_{Ca,L}$ and the related AP shortening in cAF patients. PDE8B may constitute a novel regulator of atrial $I_{Ca,L}$, with potential implications for AF pathophysiology.

Physical coupling between SERCA2 and PDE3A regulates SERCA2 activity in cardiomyocytes

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Rationale: SERCA2 controls cardiac contractility, and its activity is negatively regulated by the cAMP phosphodiesterase PDE3A through an unknown mechanism. Recent clinical trials have shown upregulation of SERCA2 gene therapy as beneficial in heart failure.
Objective: To examine whether PDE3A is physically associated with SERCA, and to further evaluate whether this protein interaction represent a novel drug target to increase SERCA2 activity.
Methods and results: PDE3 inhibition increased Ca^{2+} transients, SR Ca^{2+} load and SERCA2 activity without altering global cytosolic cAMP levels in field stimulated cardiomyocytes. SERCA2 activity was increased by PDE3 inhibition in cardiomyocytes dialyzed with 5 µmol/l cAMP by patch pipettes. Active PDE3A co-purified and precipitated with SERCA2 from left ventricular myocardium, and proximity ligation assay demonstrated co-localization of PDE3A and SERCA2 in intact cardiomyocytes. A combination of immunoprecipitation and peptide interaction experiments revealed interaction between specific cytosolic regions between amino acids 277 and 493 in PDE3A and amino acid 169 to 216 in SERCA2. By whole cell voltage clamp of intact cardiomyocytes, increased SERCA2 activity was induced by dialysis with disruptor peptides of the SERCA2-PDE3A interaction. TAT-labeled PDE3A-SERCA2 disruptor peptide fragments were further able to increase SERCA2 activity in field stimulated cardiomyocytes. PDE3A-SERCA2 disruptor peptides were able to increase SERCA2 activity in cardiomyocytes in presence of either PKI or Rp-cAMP and without concomitant phospholamban phosphorylation. Finally, PDE3A-SERCA2 disruptor peptides increased SERCA2 activity in ventricular myocytes from phospholamban-deficient mice (PLB-KO), further suggesting independence of phospholamban.
Conclusion: PDE3A is physically associated to SERCA2, and this direct interaction regulates SERCA2 activity in cardiomyocytes possibly by direct regulation of SERCA2. Cell permeable disruptor peptides of the PDE3A-SERCA2 protein-protein interaction is able to increase SERCA2 activity and may potentially offer a new therapeutic approach in chronic heart disease.

Modifier genes in the LQT1 syndrome: mechanistic analysis of NOS1AP polymorphism.

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Background: Recently, minor SNP variants of the NOS1AP gene have been reported to be associated with QT prolongation and increased incidence of sudden death in LQT1 patients. The NOS1AP gene encodes for CAPON protein that localizes NOS1 close to the sarcoplasmic reticulum (SR). NOS1 activity accounts for NO-mediated modulation of I_{CaL}, RyR2 channels and SERCA, thus interfering with regulation of Ca^{2+} handling and SR stability. Therefore we hypothesize that NOS1AP SNPs might affect NOS1 localization/function to decrease SR stability. In this setting, mutation-induced QT prolongation would induce Ca^{2+} overload, whose proarrhythmic effect would be unveiled by abnormal NOS1 localization/function.
Aim: To evaluate the effect of changes in NOS1 activity on SR functional stability, repolarization and arrhythmogenesis in the context of I_{Ks} deficiency (LQT1).
Methods: In guinea-pig ventricular myocytes subjected to I_{Ks} blockade (to reproduce the LQT1 phenotype) and adrenergic stimulation (isoproterenol, ISO), we measured electrical activity and evaluated SR functional stability, in basal condition and under selective inhibition of NOS1 (SMTC 3µM).

Results: Under basal conditions, NOS1 inhibition prolonged AP duration (APD) (128.3±7.6 ms vs 100.7±8.8 ms; 27.5%. p<0.03) enhanced I_{CaL} density (peak current density at +10 mV SMTC vs ctr: -16.6±1.2 pA/pF vs -13.5±1.0 pA/pF; p<0.05) and did not affect I_{Ks} (tail current density SMTC vs ctr: 2.3±0.5 pA/pF vs 2.7±0.7 pA/pF) and I_{Kr} (tail current density SMTC vs ctr: 0.83±0.09 pA/pF vs 0.84±0.07 pA/pF). ISO (1nM) induced delayed afterdepolarizations (DADs), an index of SR instability, in SMTC treated cells, but not in control ones (6 out of 9 treated cells; p<0.05).
Conclusions: These results indicate that NOS1 deficiency may contribute to APD prolongation and enhance Ca^{2+} influx; moreover, these effects may compromise SR stability in the presence of adrenergic stimulation. Therefore, the effects of NOS1 inhibition are such as to account for the arrhythmogenic effect of NOS1AP polymorphism.

Loss of myocardial nNOS begets atrial fibrillation by abolish the physiological right-left action potential duration gradient in human and mouse atrial myocytes

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Rationale: In both humans and several mammalian models, right to left action potential duration (APD) gradient (APD in right atria (RA) is longer than that in left atria (LA)) has been reported to ensure that impulses from both LA and RA reach the atrioventricular (AV) node at the same time given that the earlier reception of electrical impulses from the sinoatrial node in RA then LA. This action potential synchronization therefore plays a key role in maintaining the physiological heart rhythm. Recent evidences indicate that loss of this synchrony is another hallmark of onset of atrial fibrillation (AF) by associating with both ectopic beats origination and re-entry. However, the mechanisms underlying this phenomenon and its importance in the begetting of AF are still unclear.
Methodology: Whole-cell current and voltage patch clamps were used to record APs and ion currents in both human and mouse LA and RA myocytes. AF was induced in isoflurane anaesthetised mice by using trans-oesophageal electrical stimulation.
Results: Both human and mouse atrial myocytes exhibit normal RA-LA APD gradient under physiological conditions. Investigations of the ionic mechanism show that a smaller L-type calcium current ($I_{Ca,L,}$ N=10,n=22 in LA vs N=5,n=8 in RA, P<0.05) and a larger inwardly rectifying potassium current (I_{K1}, N=2,n=7 in LA vs N=6, n=14 in RA) in LA than RA are key contributors to this APD gradient in mice atrial myocytes. Whereas disruption of nitric oxide synthase (nNOS) either by acute inhibition (100nM SMTC, N=3, n=9 in both groups; P=0.1762) or gene deletion ($nNOS^{-/-}$, LA: N=3, n=15 vs RA: N=9, n=28; P=0.1788) erase this phenomenon. In addition, computer modelling illustrates that abolishing this gradient (caused by loss of nNOS) promotes rotor stability between RA-LA junctions and is associated with its arrhythmogenic consequences. Indeed, $nNOS^{-/-}$ mice displays a more then 2-fold increase in AF inducibility (N=18 per genotype, P<0.05) measured by in vivo atrial burst pacing. Further investigation indicates that SMTC, abolishes differences in $I_{Ca,L}$ (LA: N=10, n=26 vs RA: N=6, n=9; P=0.45) and I_{K1} (LA: N=2, n=6 vs RA: N=5, n=11; P=0.64) between two atriums, is responsible for the disruption of the physiological right-left APD gradient in mouse atrial myocytes.

Conclusions: Taken together, our findings identify a novel role of nNOS in atrial physiological APD gradient. Loss of nNOS is an important factor in begetting AF by abolishing this gradient through mediating $I_{Ca,L}$ and IK_1 differently within two atriums.

Measuring electrical conductivity of cardiac T-tubular systems

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Introduction: The transverse axial tubular system (TATS) propagates the action potential to the core of cardiomyocytes, triggering Ca^{2+} release also in the deepest parts of the cell. TATS structural alterations are generally associated to cardiac pathological settings and, more recently, additional electrical defects of TATS have been observed. Moreover, electrical defects are linked to non-homogeneous Ca^{2+} release and delayed myofilaments activation, significantly contributing to mechanical dysfunction.

Purpose: Structural and ultrastuctural alterations of TATS can modify the conductivity of the system. Here, we aim at studying if structural remodelling can represent the source of electrical resistance changes in TATS.

Methods: We employ fluorescence recovery after photo-bleaching (FRAP) microscopy to probe the diffusional properties in TATS lumen of isolated cardiomyocytes. Fluorescent dextran that freely diffuses from extracellular space to TATS, is used to stain T-tubules lumen of cardiomyocytes from different rodent models. The fluorescent molecular probe inside TATS lumen is then photo-bleached and the diffusion of unbleached fluorescent dextran from the extracellular space into TATS is monitored using confocal imaging.

Results: We designed a mathematical model, in which the apparent diffusion of dextran inside TATS lumen is directly correlated to the geometrical properties of the TATS. Then, inspired by a geological article (Klinkenberg, GSA Bulletin, 1951), we exploited the analogy between electrical conductivity and diffusion, eventually attempting to measure the electrical resistance of TATS. First, we validated the method using acute detubuled cardiomyocytes and then we applied FRAP microscopy to probe the electrical conductivity in Spontaneously Hypertensive Rats with overt heart failure (SHR/HF).

Conclusion: We demonstrated that TATS geometry is crucial for the electrical conductivity of T-tubules and structural and ultra-structural alterations can affect the electrical resistance of the T-tubular system.

The transcription factor NFIX: a novel modulator of cardiac rhythm in the adult heart

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Nfix is a transcription factor involved in the development of various organs. In particular, in skeletal muscle Nfix induces the switch from embryonic to fetal myogenesis. Here we investigated for the first time the expression profile and role of Nfix in the heart.

We found that NFIX is expressed in the adult sinoatrial node (SAN), ventricles and atria (SAN<ventricles<atria) at higher levels than in skeletal muscle. Nfix expression shows a double peak of expression, the first around E12.5 when cardiac chambers form and the second at birth.

In order to study the role of Nfix in the heart we compared wild-type and NFIX-knockout (KO) mice. Since no apparent morphological alterations are visible we recorded electrocardiograms in free-

moving mice. Preliminary data show that NFIX-KO mice have a higher heart rate (day 577 bpm; night 593 bpm) than age-matched WT mice (day 514 bpm; night 554 bpm). Because of the limited number of adult NFIX-KO mice, we carried out *in vitro* experiments on neonatal rat ventricular cardiomyocytes infected with a virus carrying a shRNA able to silence specifically NFIX. NFIX-silenced cardiomyocytes show faster action potential rate (2.03±1.45 Hz, n=4) than non-silenced control cardiomyocytes (0.76±0.56 Hz, n=7).
HCN channels are key regulators of heart rate; we thus evaluated their expression levels in the heart of WT and KO mice and, in agreement with the faster beating rate observed both *in vitro* and *in vivo*, we found an upregulation of HCN4 in the SAN and of HCN2 in ventricles, of NFIX-KO mice.
We also evaluated the role of Nfix in maintaining structural properties of the heart. We compared the expression levels of several genes by quantitative PCR but found no differences in the cardiac myosins (MYH6, MYH7, MLC2V), troponin (cTNI) and NKX2.5 between wild-type (n=3) and NFIX knockout (n=3) mice, indicating that the sarcomeric structure is unaltered.
In conclusion our data suggest that in the heart NFIX is a novel factor that contributes to set the heart rate by modulating HCN channels even tough further studies are necessary to properly clarify the detailed molecular mechanisms.

The coxsackievirus and adenovirus receptor regulates calcium homeostasis in the developing heart

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The coxsackievirus and adenovirus receptor (CAR) is a cell adhesion protein of the Ig superfamily which serves as receptor for different coxsackie- and adenoviruses. CAR is strongly expressed throughout the developing heart and in contrast, during adulthood CAR is only concentrated found at the intercalated discs. Surprisingly, a strong re-expression of CAR was found in patients suffering from dilated cardiomyopathy. CAR knockout (KO) mouse models revealed extensive malformations of the heart which lead to death between embryonic days E12 and E13.5 indicating an important function of CAR during heart development. Conditional CAR KO mouse models showed impairment during excitation conduction in the mature heart. The aim of this study was to investigate the physiological function of CAR during early heart beats with regard to intercellular communication and Ca^{2+} cycling in embryonic cardiomyocytes. By using a global CAR KO mouse model, the investigation of cultivated E10.5 CAR KO cardiomyocytes and E10.5 CAR KO hearts revealed a significant higher spontaneous beating frequency. Calcium imaging recordings of Ca^{2+} transients in CAR KO cardiomyocytes showed a significant faster systolic Ca^{2+} decline compared to wildtype. The analysis of the cardiac Ca^{2+} extrusion mechanism revealed a higher activity for NCX and SERCA2 in CAR KO cardiomyocytes. Gene expression and protein level of both NCX and SERCA2 was not changed in CAR KO hearts. Surprisingly, the protein level of connexin 40, 43 and 45 was decreased in CAR KO hearts. Investigation of intercellular cell coupling between neighbouring cardiomyocytes revealed for CAR KO cardiomyocytes increased dye spreading. Colocalisation of CAR with Cx45 and ZO-1 suggests that CAR is involved in a larger protein complex at the junctional sites. There, CAR may promote correct localisation of Cx45 and ZO-1 and cell-to-cell coupling. Taken together, CAR regulates intercellular communication between embryonic cardiomyocytes, is able to influence spontaneous Ca^{2+} cycling and is therefore an important regulator for early, embryonic heart beating.

Optogenetic modulation of cardiomyocyte excitability

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Introduction:

Optogenetics - the use of light-activated proteins to modulate cellular behaviour - is a fast-developing technology, first applied to neuroscience research about ten years ago. A number of studies have begun to transfer the optogenetic approach to cardiovascular research, with a focus on electrical regulation of specific cell types in the heart. There is demand to develop suitable tools to selectively trigger or silence electrical activity in cardiomyocytes (CM) and to interfere with membrane currents in non-myocytes (NM) to advance our understanding of heterocellular electrotonic coupling *in vitro* and *in vivo*. We here report on cell-type specific activation of co-cultured CM and NM using the light-gated cation channel ChR2. Furthermore, we tested three anion-selective channelrhodopsins (ACR) for their potential to inhibit action potential initiation and propagation [1, 2].

Experimental approach and preliminary results:

Neonatal hearts of the lines WT1-VSFP$^{+;+}$, αMHC-VSFP$^{+;+}$ and WT1-ChR2-H134R$^{+;+}$ were isolated and digested. Cultured cells were transfected with cDNA of ACRs coupled to fluorescent marker proteins. In order to analyse heterocellular coupling we performed whole-cell patch-clamp recordings on CM cocultured with NM from the WT1-ChR2-H134R$^{+;+}$ line (external buffer: pH 7.4; [Cl^-] 153 mM, internal solution: pH 7.2; [Cl^-] 52mM). In a first set of experiments, pulsed ChR2 activation in NM evoked action potentials in patched CM, indicating direct electrical coupling between both cell types. Next, we tested three different ACR for their potential to hyperpolarise cardiac cells (GtACR1-eGFP, iC++ mCherry and Phobos-mCherry) [3, 4]. Transfected cells were patch-clamped in the current-clamp mode to directly follow light-induced changes in membrane voltage (external buffer: pH 7.4; [Cl^-] 153 mM, internal solution: pH 7.2; [Cl^-] 12 mM). Notably, ACR activation lead to hyperpolarisation in isolated cardiac NM and to depolarisation in CM, in line with the prediction of chloride reversal potentials from the Nernst equation.

Outlook:

We would like to inhibit cardiac electrical activity by optogenetic hyperpolarisation of NM. As we have shown ACR photocurrents depolarise CM and even evoke action potentials, different to the inhibitory effect reported in neurons [1, 2, 4]. We expect that NM, which are connected to CM, have more negative potentials, resulting in depolarising ACR currents also in NM. Therefore, we are looking for a stronger tool to inhibit cardiac activity and plan to generate a light-activated K^+-channel.

References:

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Variability in the kinetics of cardiac Ina

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We aimed to establish the existence of variability in the kinetical parameters of cardiac I_{Na}, distinguish it from experimental noise, and shed light on the possible range and distribution such variability might take.

Our study had two parts: First, we conducted a meta-analysis of the midpoints of activation and inactivation reported in over 100 patch-clamp experiments on wild-type SCN5A in expression systems. Here we investigated the correlation between reported midpoints of activation and of inactivation, and made a worst-case estimate of the variability that could be introduced by differences in experimental protocol and conditions. Next, we performed patch-clamp experiments under controlled conditions wherein we measured the current elicited by a single step from -120mV to -20mV. Using model cell experiments and arguments from identifiability theory we hypothesized that the time constants of inactivation could be obtained consistently and accurately, making them good candidates to measure any cell-to-cell variability.

In our meta-analysis, we found a very wide range of reported values, with midpoints of activation between -60mV and -30mV all falling within two standard deviations of the mean. For inactivation, this range stretched from -110mV to -60mV. A strong correlation between midpoint of activation and inactivation was seen, with an average distance of 40mV between the midpoints. Removing this linear component showed that midpoints of inactivation still varied over a range of 20mV. This was equivalent to a *worst-case* estimate of the variance introduced by different experimental conditions, making it unlikely that this is purely a measurement artifact.

In the single voltage-step experiments we observed variance in the time constants of inactivation, obtained either via direct fits of bi-exponentially decaying curves or by fitting a full model. For both slow and fast inactivation, time constants varied between half and double the mean value for all cells. By performing repeated fits (with randomly chosen starting positions) to the same data, we showed that the difference in time constants found between cells was considerably larger than the variance introduced by uncertainty in the fitting method.

We conclude there is a strong suggestion of variability between the midpoints of activation and inactivation of I_{Na} in expression systems, and clear evidence of variance between individual cells' time constants of inactivation.

Our work provides an experimental foundation for the cell-to-cell variability already hypothesized in simulation studies. This is a vital step in characterizing and understanding the role of inter-subject variability in the genesis and treatment of cardiac arrhythmia.

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