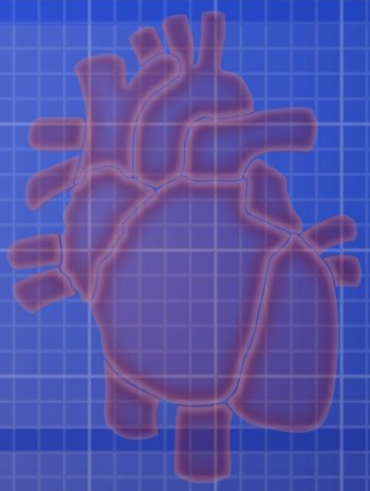
DCR Cluster for Cardiovascular Research



Networking Symposium Program

27 MAY 2016
Murtenstrasse 35, H-Floor

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Contact:
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maguy@pyl.unibe.ch

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Acknowledgements

Dear Participants, Dear Guests,

It is our great pleasure to welcome you to the first Networking Symposium of the DCR Cluster for Cardiovascular Research.

The DCR Cluster for Cardiovascular Research commenced activities in early 2015 with the aims of promoting and strengthening research collaborations between DCR and UniBE researchers, raising visibility and awareness of cardiovascular research in Bern, enriching the training environment of junior researchers, as well as providing a framework and logistical support for multi-team and/or interdisciplinary projects.

The aims of this symposium are to stimulate interactions and promote collaboration among cardiovascular research teams at the University of Bern, to support and encourage young and promising researchers working in the field of cardiovascular research, and to raise awareness of ongoing cardiovascular research and research interests among scientists and clinicians at the University of Bern.

We would like to thank the organization committee, Ms M Abdelhafez, Ms M. Arnold, Ms. N. Méndez Carmona, Ms S. Vermij, and Ms R. Wyss, for volunteering their time and for their enthusiasm, as well as Dr. D. Rodriguez for the graphic design. Importantly, we would also like to express our gratitude for the generous sponsorship provided by the institutions mentioned below – without their support this event would not have been possible.

Sincerely,

Dr. phil. nat. Ange Maguy

PD Dr. phil. nat. Sarah Longnus

Program

7:30-7:55:	**Registration and speaker preparation**
7:55-8:00:	**Welcome and introduction**
8:00-8:50:	**Keynote Speaker 1: Prof. Colleen Clancy** ***Predictive in silico pharmacology: From atom to rhythm***
8:50-9:10:	**Prof. E. Niggli:** ***Cardiac EC-coupling and Ca^{2+} signaling: Regulation and pathophysiology***
9:10-9:30:	**Prof. H. Abriel:** ***Cardiac channelopathies***
9:30-9:50:	**Prof. S. Rohr:** ***Cardiac fibroblasts - more than passive bystanders in arrhythmogenesis?***
9:50-11:20:	**Combined coffee break and poster session**
11:20-12:10:	**Keynote Speaker 2: Dr. Sean Davidson** ***Protecting the heart from ischaemia and reperfusion injury***
12:10-12:30:	**Prof. R. Rieben:** ***Role of the vascular endothelium and the plasma cascade systems in ischemia/reperfusion injury***
12:30-12:50:	**Dr. C. Zuppinger:** ***3D cell culture for cardiovascular research and drug development***
12:50-13:10:	**PD Dr. M. Heller:** ***Quantitative profiling of circulating plasma microparticle associated proteins by mass spectrometry***
13:10-13:20:	**Poster awards & Sponsor recognition**
13:20-14:30:	**Lunch break (lunch not provided)**
14:30-17:00:	**Laboratory open house**

Keynote Speakers

Prof. Dr. Colleen E. Clancy

University of California Davis, School of Medicine, Davis, CA

The Clancy Lab engages in research involving 1) computational and mathematical modeling of the heart and brain to reveal mechanisms of disease; (2) computational pharmacology for applications in research, regulation and clinical application; (3) modeling and simulation of arrhythmia and other disease mechanisms. Scientific work from her lab has been featured in Scientific American and BBC radio.

Dr. Clancy has authored 70+ publications, serves on the editorial board for the Journal of Physiology, the advisory board of the National Biomedical Computation Resource, as a member of the NIH Multiscale Modeling Consortium, the Heart Rhythm Society Fellowship Subcommittee, and engages in peer review for dozens of national and international granting agencies and journals. In the past year Dr. Clancy led a multidisciplinary team of junior and senior investigators to develop two distinct NIH projects on the topic of computational pharmacology that were both scored in the top 1% of all grants reviewed.

Dr. Sean Davidson

The Hatter Cardiovascular Institute, University College London

Sean Davidson obtained his PhD in Melbourne, Australia on the regulation of heat shock proteins (HSPs) and protection from stress. During a postdoctoral position at the École Normale Supérieure in Paris, he demonstrated that certain members of this protein family called “small heat shock proteins” are also essential for early cardiac development. He moved to London in 1998 and is now a Senior Research Fellow at the Hatter Cardiovascular Institute at University College London, where he works on developing methods to protect the heart from ischaemia and reperfusion injury. In the last few years, through a grant from the Medical Research Council, he has been exploring the potential intracellular signalling role of endogenous nanoparticles called exosomes, as well as their role in cardioprotection. Via this circuitous route, his research has returned full circle to where it began, on the importance of heat shock proteins – which turn out to be major components of exosomes.

List of Posters

Nb.	Name	Title of poster
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P3	Beatrice Bianchi	Genetic mutations of the cardiac channel TRPM4: an overview
P4	Joaquim Blanch	Inositol-1,4,5-triphosphate signalling in cardiomyocytes
P5	Morgan Chevalier	Regulation of the cardiac sodium channel $Na_v1.5$ by CASK is mediated by calcineurin
P6	Emilie Farine	Controlled reperfusion strategies improve cardiac recovery after global, warm ischemia in an isolated working rat heart model of donation after circulatory death (DCD)
P7	Carolina Garcia Poyatos	Zebrafish's OXPHOS system during homeostasis and heart regeneration
P8	Echrack Hichri	The activation of the sodium current is potentiated by ephaptic effects in a high resolution mathematical model of the intercalated disc
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P10	Duilio Michele Potenza	Protein Phosphatase-1 Increases Calcium Spark Frequency in Murine Cardiomyocytes via Modulation of RYR2 Phosphorylation
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P12	Andres Sanz Morejon	Novel strategies for the study of fibrosis regression during zebrafish heart regeneration
P13	Marcel Wullschleger	Local crosstalk of inositol 1,4,5-trisphosphate receptor- and ryanodine receptor-dependent Ca^{2+} release in atrial myocytes
P14	Rahel Wyss, Natalia Méndez Carmona	Tolerance of cardiac mitochondria and vascular endothelium to ischemia and reperfusion

P15	Miguel Fernandez Tenorio	Opposite Changes Of Ca^{2+} Wave Threshold And Fractional SR Ca^{2+} Release During SERCA Stimulation In Cardiomyocytes
P16	Radoslav Janicek	Calcium uncaging with visible light
P17	Silvan Jungi	S100A1 AAV-based gene transfer improves hemodynamic recovery from global ischemia-reperfusion injury in an isolated working rat heart model
P18	Stéphanie Lecaudé	Cardiac microRNA expression and release are regulated during early reperfusion after global ischemia in an isolated rat heart model of donation after circulatory death (DCD)
P19	Ines Marques	Cardiac regeneration in zebrafish: a role for LOX
P20	Théo Meister	Assisted reproductive technology increases the vasoconstrictor responsiveness to angiotensin II in the aorta
P21	Lijo Cherian Ozhathil	TRPM4 variants in childhood atrio-ventricular block
P22	Maria Nieves Sanz Garcia	Generation, validation and characterization of an adult-inducible cardiac-specific SIRT1 knock-out mouse model
P23	Urs Thomet	Remake of Cardiac Safety Assessment – HERG Discontinued?
P24	Adrian Segiser	Atrophic remodeling limits contractile function of long-term unloaded hearts assessed via ex vivo whole heart perfusion

No.	Lab	Location	Keywords	Timing info
1	Channelopathies	Murtenstrasse 35, room G801	Ion channel disorders, ion channel regulation, biochemistry, electrophysiology	Open for entire duration (14.30-17.00)
2	Cardiovascular Surgery Research	Murtenstrasse 35, rooms C812 & C806 +	Heart transplantation with donation after circulatory death, cardiac unloading, reperfusion injury, graft evaluation, energy metabolism	Open for full duration (14.30-17.00)
3	Cardiovascular research, ischemia/ reperfusion	Murtenstrasse 50. Meeting place: entrance lobby (badge is required)	I/R injury, endothelial cells, plasma cascades: complement and coagulation, surgical models	14.30-15.30 (first round); 16.00-17.00 (second round) Maximum 5 persons per round
4	Computer Simulation and Experimental Investigation of Impulse Formation and Conduction in Cardiac Tissue	Bühlplatz 5, rooms 147a and 109. Meeting place: entrance lobby *	Cardiac action potential, ion channels, mathematical modeling, cardiac cell cultures, microelectrode arrays	First round: 14.30-15.30; second round: 16.00-17.00. Maximum 6 persons per round
5	Laboratory of cellular optics	Bühlplatz 5, -113	Heterocellular electrotronic interactions between cardiomyocytes and myofibroblasts; multisite optical recording of transmembrane voltage; patterned growth cell cultures; SICM; MEA recording	Open for the entire duration (14.30-17.00) Demonstrations depending on availability of cell cultures
6	Cardiac calcium signaling lab	Bühlplatz 5, rooms 25-26	Cardiac cellular calcium signaling; ryanodine receptor phosphorylation; confocal microscopy, patch-clamp; photolysis of caged compounds	Open for the entire duration (14.30-17.00)
7				

* Wheelchair access at service door and elevator; contact Jan Kucera if required.

\+ Badge access only; please call 22686 to enter; phone located on c-floor across from elevators.

Campus Map


Open house locations
Bühlplatz 5
4, 5, 6, 7
Murtenstrasse 50
3
Murtenstrasse 35
1, 2
Bühlstrasse
Freiestrasse
Gertrud-Woker-Strasse
Baltzerstrasse
Sahlistrasse
Depotstrasse
Stadtbachstrasse
Finkenrain
Finkenhubelweg
Hochbühlweg
Bühlstrasse
Niesenweg
Lindenrain
Stadtbachstrasse
Bühlstrasse
Ristorante Azzurro
Freiburgstrasse
Inselspital Hôpital universitaire de Berne
BEKB
Friedbühlstrasse
Satellite
Google

Poster Abstracts

P1. Effect of C1-INH on vascular injury and plasma cascade activation in a porcine limb amputation and reperfusion model

Mai M Abdelhafez [1,2], Jane Shaw[1], Damian Sutter, MD, [3] Jonas Schnider, MD, [3] Hansjörg Jenni, [4] Esther Voegelin, MD, [3] Mihai A. Constantinescu, MD, [3] and Robert Rieben, PhD[1]

[1] Department of Clinical Research, University of Bern, Bern, Switzerland. [2] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland. [3] Clinic of Plastic and Hand Surgery, University Hospital, Bern, Switzerland. [4] Clinic of Cardiovascular Surgery, University Hospital, Bern, Switzerland

Background: Trauma is the main cause of lower limb amputation in developing countries. Revascularization of the amputated limb within 4 – 6 hours is essential to avoid extensive reperfusion injury leading to vascular leakage, edema and tissue necrosis. Ischemia reperfusion (I/R) injury is a pathological inflammatory condition that occurs during reperfusion of an organ or tissue after prolonged ischemia. Vasculoprotective pharmacological intervention might be a solution to prolong the time window between amputation and successful replantation. We used the C1-easterase inhibitor (C1-INH) in this study because of its known inhibitory effects on activation of the complement, coagulation and kinin cascades.
Methods: 15 wild type porcine forelimbs were amputated and ex vivo perfused for 12 hours with autologous blood using a purpose built extracorporeal perfusion circuit after exposure to 9 hours of cold ischemia at 4°C. The treated limbs were passively perfused with 500 ml hydroxyethyl starch solution (HAES) containing C1-INH (1 U/ml) after 2 hours of cold ischemia (treatment group, n=8), while the non-treated limbs (control group, n=7) were rinsed with the same amount of HAES without C1-INH. Tissue samples at baseline and at the end of perfusion were stained for markers of endothelial cells (EC) as well as activation of complement, coagulation and kinin by immunofluorescence. Blood samples at baseline and different time points during perfusion were tested for inflammatory and complement markers by ELISA and multiplex suspension array.
Results: As assessed by immunofluorescence there was a significant decrease in tissue deposition of IgM ($P = 0.009$), IgG ($P = 0.01$), C3c ($P = 0.0011$), C5b-9 ($P = 0.0059$), and fibrin ($P < 0.001$) in the treatment group compared to the control group. Moreover the treatment group expressed higher levels of EC markers CD31 ($P = 0.0006$) and VE-Cadherin ($P = 0.006$) than the control group. The bradykinin in plasma ($P = 0.03$, after 6 hours of perfusion) and expression of bradykinin receptor 1 ($P = 0.01$) were increased in the control group compared to baseline, but the expression of bradykinin receptor 1 was not significantly different between treatment and control group. Hepatocyte growth factor (HGF) in plasma was significantly elevated compared to baseline in the treatment group ($P = 0.008$) whereas this was not the case in the control group. Edema formation, compartment pressure, CK-MM, C5a, sC5b-9, IL-6 and MCP-1 production showed no differences between treatment and control group.
Conclusion: Skeletal muscle reperfusion injury after 9 hours of cold ischemia leads to severe damage that was seen as edema and high compartment pressure as well as high levels of immunoglobulin deposition, EC damage, complement, kinin activation and fibrin deposition. The use of the cytoprotective drug C1-INH helped to protect the vascular endothelium and the muscle tissue from deposition of immunoglobulins, complement and fibrin. However, C1- INH had no significant effect on edema, CK-MM, presence of C5a and sC5b-9 and the pro-inflammatory cytokine production. This might be explained by the closed ex vivo circuit with plastic tubing, filters from the oxygenator and the absence of the liver.

P2. Molecular mechanisms of cardioprotective reperfusion strategies in an isolated rat heart model of donation after circulatory death

M. Arnold, N. Méndez Carmona, R. Wyss, E. Farine, P. Niederberger, A. Segiser, H. Tevaearai Stahel, T. Carrel, S. Longnus

Clinic of Cardiovascular Surgery, Inselspital, Bern University Hospital and Department of Clinical Research, University of Bern

Background:
Donation after circulatory death (DCD) could significantly improve donor heart availability, which is a critical problem in heart transplantation. Unlike conventional donor organ donation following brain death, organs obtained with DCD undergo a period of warm ischemia. Warm ischemia can rapidly damage the heart, and therefore strategies to limit damage and/or improve heart recovery are urgently needed. Because interventions are limited prior to graft procurement for ethical reasons, therapies applied at the time of reperfusion have the potential to provide an important clinical impact. Key intracellular signaling pathways, such as the reperfusion injury salvage kinase (RISK) pathway or that of 5'AMP-activated protein kinase (AMPK), are implicated in regulating energy substrate metabolism and could also be involved in the cardioprotective reperfusion strategies.
Previous work from our lab identified several reperfusion strategies to be cardioprotective in terms of hemodynamic recovery.
Therefore, we investigated the activation of key signaling molecules that potentially underlie these cardioprotective reperfusion strategies, in order to identify new pharmacologic targets.
Methods:
Experiments were performed using an isolated, working rat heart model of DCD. Hearts of male Wistar rats underwent 20 min baseline perfusion, 27 min global, normothermic ischemia and different periods of reperfusion (3, 5, 10, 15 min). During aerobic perfusions, hearts were perfused with modified Krebs-Henseleit bicarbonate (KHB) buffer containing 11 mM glucose and 1.2 mM palmitate. Mechanical postconditioning (MPC; 2x30sec), mild hypothermia (MH; 30°C, 10min), and hypoxia (HY; no O2, 2min) were applied at the onset of reperfusion and compared with controls (no strategy). Phosphorylation of AMPK, acetyl-CoA carboxylase (ACC) and components of the RISK pathway including Akt, Akt substrate of 160 kDa (AS160) and glycogen synthase kinase 3 beta (GSK3β) were measured by western blot at 3, 5, 10 and 15 minutes reperfusion.
Results:
No differences in hemodynamic baseline parameters between the different groups were observed. Initial data indicates a greater Akt phosphorylation following MPC compared to control at 10 and 15 minutes reperfusion. No differences were obvious at earlier time points. This phosphorylation pattern seems to correspond with the downstream elements of Akt, AS160 and GSK3β. Preliminary data for AMPK pathway activation were also gathered. A high phosphorylation / activation of AMPK was measurable after 27 minutes of ischemia, whereas at 3 minutes of reperfusion the phosphorylation levels were already decreased again for all four groups (control, MPC, MH and HY) whereby HY presented the highest AMPK phosphorylation from all the groups.
Conclusions:
Key intracellular signaling pathways seem to be activated by cardioprotective reperfusion strategies. These activations appear to play a role in the very first minutes after ischemia for the AMPK pathway, but only after 10 to 15 minutes of reperfusion for the RISK pathway. Identification and understanding of molecular changes with cardioprotective reperfusion strategies could help improving cardiac functional recovery of DCD hearts.

P3. Genetic mutations of the cardiac channel TRPM4: an overview

Bianchi B.[1], Ozhathil L.[1], Syam N.[1;2], Rougier J.S.[1], Amarouch Y[1;3], and Abriel H.[1].

[1] Department of Clinical Research, and Swiss National Center of Competence in Research (NCCR) TransCure, University of Bern, Switzerland
[2] Department of Cellular and Molecular Medicine, KU Leuven, Belgium
[3] MNSEM Laboratory, University Sidi Mohammed Ben Abdellah, Fez, Morocco

TRPM4, a non-selective calcium-activated cation channel has been known for its decisive role in electrical activity of heart. Dominantly inherited mutations in TRPM4 gene are associated with severe cardiac disorders like isolated cardiac conduction diseases (ICCD), Atrioventricular Block (AVB) and Brugada Syndrome.
Despite many new findings regarding TRPM4 role in cardiac conduction, the mechanisms underlying TRPM4-dependent conduction disorders are not fully understood.
In this work, we characterized **20** TRPM4 genetic variants found in patients using biochemical and electrophysiological methods: HEK293 cells were transiently transfected with the mutated TRPM4 gene and after 48h, cells were harvested and membrane expression was evaluated, together with whole-cell patch clamp to characterize the mutation phenotype.
Recently, three of these variants were characterized in depth for their interesting role in cardiac conduction disorders: I376T variant has been found in 39 relatives of a large French fourth generation pedigree which had led to progressive familial heart block type I (PFHBI). Western blot and cell surface biotinylation experiments observed an increase in expression of TRPM4-p.I376T at the cell membrane and an increase in current density as observed with whole-cell configuration of patch clamp studies corroborated with expression studies.
A432T, G582S and the double mutation A432T/G582S have been found in childhood AVB patients. A432T and A432T/G582S showed decreased expression of the protein at the cell membrane, and inversely G582S variant showed increased expression. Further functional characterization of these variants using whole cell patch clamp configuration showed a loss-of-function and gain-of-function respectively. The loss-of-function TRPM4 variants are most likely caused by misfolding-dependent altered trafficking. Ability to rescue this variant with lower temperature confirmed our hypothesis. Interestingly such variants may therefore provide a novel venue for using pharmacological chaperons as treatment strategies.
In conclusion, our data further support the possible role of TRPM4 in human cardiac conduction system and cardiac conduction disorders.

P4. Inositol-1,4,5-triphosphate (IP3) signaling in cardiomyocytes

Joaquim Blanch, Marcel Egger

Department of Physiology, University of Bern, Switzerland

In the heart, the dominant mechanism of intracellular Ca^{2+} release is Ca^{2}-induced Ca^{2+}-release (CICR) via sarcoplasmic reticulum (SR) Ca^{2+} release channels (Ryanodine Receptors, RyRs). Inositol-1,4,5-trisphosphate (IP3) is produced upon agonist binding to a G-protein coupled receptor (GPCR). IP3 can trigger SR Ca^{2+} release through another type of receptor, the IP3 receptors (IP3R). The contribution and significance of IP3-induced Ca^{2+} release (IP3ICR) in cardiac excitation-contraction coupling (ECC) is still a matter of debate. Evidence suggests that IP3 signaling may be involved in a variety of cardiac pathologies (e.g. atrial fibrillation and heart failure). Pathophysiological stress promotes an increased production of IP3 and expression of its receptor. It has been reported in many studies the relation between RyR and IP3R in atrial myocytes, however, it is still unclear how IP3ICR may contribute in ventricular myocytes. Our working hypothesis is that in a physiological situation, IP3ICR fine-tunes the RyR activity and ECC in atrial myocytes and, on a plausible smaller scale, in ventricular myocytes. In a pathophysiological situation, the enhanced IP3ICR may play a modulatory role in the appearance and propagation of spontaneous or triggered Ca^{2+} events (e.g. Ca^{2+} waves) in ventricular myocytes as well as it

is known for atrial myocytes. To assess this question, the contribution of IP3ICR to the total SR-Ca^{2+} leak in mice ventricular myocytes in response to IP3 production (e.g. Endothelin-1 (ET1)) has been examined. This was assessed by a protocol adapted from Shannon et al. 2002 in combination with pharmacological inhibition of RyRs. The results show an increase of the leak of 45% with ET1 stimulation. To identify a possible contribution from PKC phosphorylation pathways, an IP3R blocker (2-APB) was used in combination with ET1. We found a significant contribution to the total SR-Ca^{2+} leak when IP3ICR was stimulated and a significantly decreased effect when 2-APB was used in combination with ET1. In our experimental conditions, ET1 lead to an increase on the SR Ca^{2+} content. To assess this question we applied a current-voltage protocol and found a shift towards more negative voltages in the presence of ET1, pointing towards a possible modification of L-type Ca^{2+} channels due to PKC stimulation, which may explain the increase in SR Ca^{2+} content. We hypothesized that this process may involve Ca^{2+} release via IP3Rs activation. In addition, local RyR Ca^{2+} release may as well modify the Ca^{2+} release properties of the IP3R. To assess this, further experiments combine state-of-the-art biophysical approaches with pharmacological interventions, electrophysiology (whole-cell configuration of the patch clamp technique), rapid confocal Ca^{2+} imaging and two-photon photolysis (TPP) of caged-compounds on acutely isolated cardiac myocytes. The general application and preliminary results of the combined approaches will be presented. Using TPP of caged IP3 or caged Ca^{2+} in combination with pharmacological separation of the RyRs and IP3Rs events we will examine details of the functional crosstalk between these two Ca^{2+} release channels and functional local distribution of IP3R.

P5. Regulation of the cardiac sodium channel $Na_v1.5$ by CASK is mediated by calcineurin

Morgan Chevalier, Sabine Nafzger, Jean-Sebastien Rougier, Hugues Abriel

Department of clinical research, University of Bern, Switzerland.

Background:
The voltage-gated Na^+ channel, $Na_v1.5$, is responsible for the rapid depolarization of the cardiac action potential (AP) and thus allows for the conduction of the electrical impulse throughout the myocardium. The channel $Na_v1.5$ is present in different membrane domains in myocyte where it interacts with specific partners such as MAGUK (membrane-associated guanylate kinase) proteins, which are key regulators of ion channels. Among this family of proteins, CASK (calcium/calmodulin-dependent serine protein kinase) has been recently shown to interact with $Na_v1.5$ at the lateral membrane of cardiomyocytes. Here, we investigate the role of CASK on the function of $Na_v1.5$ in cardiac myocytes.
Methods and results:
To assess the functional consequences of the interaction between CASK and $Na_v1.5$ channels, patch-clamp experiments were first performed in TSA-201 cells transfected with shRNA plasmids to silence the endogenous CASK expression. It was found that sodium current (I_{Na}) is twice higher in cells where CASK was silenced. I_{Na} recording were also performed in isolated cardiomyocytes from WT or cadiac specific CASK KO mice. In CASK KO cardiomyocytes, I_{Na} is increased by 40% without any significant modifications of the steady state activation and inactivation. In addition, AP recordings were performed. It was shown that AP threshold is significantly lower in CASK KO cardiomyocytes while dV/dt and resting membrane potentials are not significantly modified. All together, these data show that CASK is a negative regulator of $Na_v1.5$ channels. In order to investigate whether modulation of $Na_v1.5$ expression is involved in this process, western blot using antibodies against $Na_v1.5$ were performed in whole heart, brain, and skeletal muscle from WT or heart specific CASK KO mice. The total expression of $Na_v1.5$ is increased in the CASK KO heart compared to the WT.
Recently, it has also been shown that CASK interacts with the calcium-dependent serine-threonine phosphatase: calcineurin in cardiomyocytes and inhibits its activity. In order to assess the role of calcineurin in the CASK dependent regulation of $Na_v1.5$. I_{Na} were recorded in CASK silenced TSA-201 transfected cells. In presence of DMSO 0.1%, I_{Na} is doubled in CASK silenced cells while after 1 hour treatment with cyclosporin A 10 μM, a calcineurin inhibitor, I_{Na} remains unchanged.

Conclusion:
According to these results, calcineurin appears to be a positive regulator of Nav1.5 whose activity is inhibited by an interaction with CASK.

P6. Controlled reperfusion strategies improve cardiac recovery after global, warm ischemia in an isolated working rat heart model of donation after circulatory death (DCD)

E. Farine*, P. Niederberger*, R. Wyss, N. Méndez, T. Carrel, H. Tevaearai Stahel, S. Longnus
* equally contributed

Clinic of Cardiovascular Surgery, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

Background: Donation after circulatory death (DCD) could improve cardiac graft availability, which is currently insufficient to meet transplant demand. However, in DCD heart transplantation, organs undergo an unavoidable period of warm ischemia and most cardioprotective approaches can only be applied at reperfusion (procurement) for ethical reasons. Therefore, we investigated whether strategies applied at the onset of reperfusion may improve heart recovery after warm global ischemia.
Methods: Isolated hearts of male Wistar rats were perfused in working-mode for 20 min (baseline), subjected to 27 min global ischemia (37°C), and 60 min reperfusion (n=43). Mild hypothermia (MH; 30°C, 10 min), mechanical postconditioning (MPC; 2x30 sec), hypoxia (HY; no O_2, 2 min) and low pH (pH 6.8-7.4, 3 min) were applied at the onset of reperfusion and compared with controls (i.e. no strategy applied). Data (mean±SD) were compared using t-tests; p-values were corrected for multiple comparisons.

Results: Post-ischemic recovery was higher in MPC, MH and HY treated hearts compared to controls. No difference was measured for low pH (see Table below).

	LV Work [%]	**Cardiac Output** [%]	**$dPdt_{max}$** [%]	**O_2 cons.** [%]	**Coronary Flow** [mL/min]	**LDH release** [$U*min^{-1}*g\ wet^{-1}$]	**Cyt c release** [$ng*min^{-1}*g\ wet^{-1}$]
Contr	44±7	3±6	57±10	37±15	13±3	396±276	33±18
MH	62±7*	20±18	74±12*	55±13	14±2	112±128*	22±16
MPC	65±8*	27±19*	74±7*	61±14*	17±3*	185±140	35±15
HY	61±11*	8±16	85±20*	50±12	16±3	213±167	25±9
Low pH	45±13	12±11	60±14	44±10	15±3	251±228	35±16

All parameters are reported as 60 min reperfusion values expressed as percentage recovery of baseline, except for coronary flow, lactate dehydrogenase (LDH) and cytochrome c (Cyt c) release, expressed as the absolute value at 10 min reperfusion.
*$p<0.05$ vs control; left ventricular (LV) work (developed pressure*heart rate) / $dPdt_{max}$ (maximum contraction rate) / O_2 cons (O_2 consumption)

Conclusions: MH, MPC and HY, but not pH, seem to improve hemodynamic recovery vs controls. Reduced necrosis (MH and MPC), increased oxidative metabolism (MH and MPC), improved endothelial function (MPC) and decreased mitochondrial damage (MH and HY) may contribute to improved functional recovery. Cardioprotective strategies applied at graft procurement, could improve DCD graft recovery and limit further injury; however, optimal reperfusion strategies remain to be identified.

P7. Zebrafish's OXPHOS system during homeostasis and heart regeneration

Carolina Garcia-Poyatos, Sara Cogliati, Jose Antonio Enríquez and Nadia Mercader.

Mitochondrial activity plays a central role in cardiovascular disease(CVD) through its role as an energy and reactive oxygen species(ROS) source. They rely mainly on the oxidative phosphorylation system (OXPHOS), a system extremely conserved along evolution. According to the Plasticity Model, OXPHOS is a dynamic system composed by 5 respiratory complexes(RCs), which can be associated in larger structures called respiratory supercomplexes(SCs) to increase the efficiency of electron flux according to cell demands. While the zebrafish is a well-established animal model in CVD research, its use for mitochondrial biology has until now been limited.
Our results based on Blue Native polyacrylamide gel electrophoresis (BN-PAGE) suggest that high molecular weight SCs are reduced in zebrafish compared to mouse. Although differences in supercomplexes assembly were found between mice strains, zebrafish strains AB and WIK do not show any difference in their OXPHOS pattern. We also show that RC and SC ratio is slightly altered in response to ventricular cryoinjury.
In mammalian SCs, the protein SCAFI(Cox7a2l) has been identified as cIII and cIV assembly factor. In contrast, its homologous in zebrafish seems to not have the same function than in mice by BN-PAGE Western Blot, even though its amino acids sequence resemble the functional isoform of the mouse protein. Moreover, to study this function in zebrafish a SCAFI knock out is being performed by CrisprCas technology.
Differences in zebrafish OXPHOS pattern compared to mouse might lead to difference adaptation to energy demands and ROS production. Furthermore, OXPHOS remodeling might occur during cardiac regeneration.

P8. The activation of the sodium current is potentiated by ephaptic effects in a high resolution mathematical model of the intercalated disc

Echrak Hichri[1], Hugues Abriel[2], Jan P. Kucera[1]

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[2] Department of Clinical Research, University of Bern, Switzerland
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Background: Numerous cardiac arrhythmias result from conduction disorders in the myocardium. Recent studies proposed that electrical potentials occurring in narrow extracellular spaces such as intercalated discs may contribute to action potential propagation in addition to gap junctional coupling (ephaptic conduction). Furthermore, Na^+ channels are not uniformly distributed in intercalated discs but cluster in perinexal regions near gap junctions, leading to the hypothesis that Na^+ channel distribution in intercalated discs may modulate ephaptic intercellular interactions.
Aim: Our aim was to investigate in a mathematical model how cardiac Na^+ current (I_{Na}) dynamics are influenced by the extracellular potential (V_e) in a narrow extracellular cleft and by the distribution of Na^+ channels.
Methods: The model consisted of a narrow disc-shaped cleft discretized at a high resolution separating either one cell membrane containing Na^+ channels and a non-conductive obstacle, or two excitable membranes mimicking the situation of two cardiomyocytes separated by an intercalated disc. I_{Na} was represented using the Luo-Rudy model (Hodgkin-Huxley formalism) or using the Clancy-Rudy Markovian model. The intracellular domain of the first cell was subjected to a voltage-clamp activation protocol while the intracellular potential of the second cell was clamped to the resting potential.
Results: In the model with one membrane facing a non-conducting obstacle, decreasing cleft width resulted in decreasing I_{Na} peak intensity at voltage steps $\geq$–30 mV. This decrease was caused by the negative V_e in the cleft, which decreased the driving force for I_{Na} (self-attenuation). However, at voltage steps near threshold (–58 to –40 mV), the negative V_e caused by the earliest activating I_{Na} in the center of the disc precipitated the activation of I_{Na} in a ring-shaped peripheral region of the disc. This resulted in a

larger I_{Na} in simulations with clefts 10-200 nm wide. When total I_{Na} was considered, the negative V_e resulted in a cleft-dependent shift of the steady state activation curve to more negative potentials and a steepening of the curve. Narrowing the cleft thus lowered and accentuated the threshold of I_{Na}. These effects were more prominent in discs with a larger diameter and when the sodium channels were redistributed into a central region of the disc. Similar results were obtained with both I_{Na} models. In the two-cell model, the negative V_e caused by I_{Na} in the first cell resulted in activation of I_{Na} in the second cell after a short delay (~1 ms) for clefts <70 nm, even when the intracellular potential of the second cell was held at resting potential.
Conclusions: These results indicate that the V_e caused by I_{Na} in a narrow restricted extracellular space exerts a major feedback on Na^+ channel behavior, modulating the voltage-dependence of activation, the threshold behavior and the kinetics of I_{Na}, and thus cellular excitability. These effects are strongly influenced by the spatial distribution of Na^+ channels. These phenomena may be instrumental in determining ephaptic interactions and conduction, and our findings contribute to understand the complex spatiotemporal behavior of currents and potentials in the intercalated disc and the physiology of cardiac excitation.

P9. High circulating fatty acids prior to warm ischemia decrease cardiac recovery in an isolated rat heart model of donation after circulatory death

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Background: Insufficient cardiac graft availability could potentially be improved with donation after circulatory death (DCD). Preclinical studies suggest that high pre-ischemic levels of circulating fatty acids, as may expected with DCD, affect post-ischemic cardiac recovery. Therefore, we investigated whether acute cardiac exposure to high levels of fatty acids prior to global warm ischemia alters subsequent recovery.
Methods: Isolated hearts of male Wistar rats underwent 20 min baseline working-mode perfusion with glucose (11 mM) and either high fat (1.2 mM palmitate; HF) or no fat (NF), followed by 27 min global ischemia (37°C), and 60 min glucose only reperfusion (n=16). Additional hearts underwent 10 min reperfusion with radiolabelled ([U-^{14}C]- or [5-^{3}H]-) glucose for measurement of glucose oxidation (GOX) and glycolysis (GLY; n=5-7). A separate series of hearts (NF/HF) were stopped at various time points to measure glycogen content. Release of lactate and cytochrome c was also monitored. Data (mean±SD) were compared using t-tests; p-values were corrected for multiple comparisons.
Results: After 60 min reperfusion, percent recovery of rate-pressure product (peak systolic pressure*heart rate) was two-fold lower in HF vs NF hearts (31±17% vs 69±17% baseline; $p<0.01$). Trends toward lower GLY and GOX rates, with a significant reduction in GOX at 10 min reperfusion was measured HF vs NF hearts. Correspondingly, HF hearts resulted in a greater imbalance between GLY and GOX during early reperfusion compared to NF hearts. Furthermore, lactate (10±2 vs 6±2 µmol*g tissue^{-1}; $p<0.05$) and cytochrome c release (18±9 vs 5±2 ng*min^{-1}*g wet^{-1}; $p<0.01$) were greater in HF vs NF hearts at 10 min reperfusion. Glycogen consumption during ischemia was not different between groups.
Conclusion: Acute pre-ischemic exposure of hearts to high fat significantly decreases hemodynamic and metabolic recovery upon reperfusion compared to no fat. Decreased coupling between glycolysis and glucose oxidation in HF conditions likely contributes to lower hemodynamic recovery through exacerbation of ischemia-reperfusion injury. Thus, pre-ischemic circulating fatty acid levels should be taken into consideration in pre-clinical models and clinical situations involving cardiac ischemia-reperfusion. In the context of DCD, pre-ischemic interventions are limited, but optimizing energy substrate metabolism at the time of procurement may facilitate use of these hearts.

P10. Protein Phosphatase-1 Increases Calcium Spark Frequency in Murine Cardiomyocytes via Modulation of RYR2 Phosphorylation

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Changes in cardiac ryanodine receptor (RyR2) phosphorylation are thought to be important regulatory and disease related post-translational protein modifications. The extent of RyR2 phosphorylation is mainly determined by the balance of the activities of protein kinases and phosphatases, respectively. Increased protein phosphatase-1 (PP1) activity has been observed in heart failure (HF), but the regulatory role of this enzyme on intracellular Ca2+ handling remains poorly understood. To determine the physiological and pathophysiological significance of increased PP1 activity, we investigated the effect of the PP1 catalytic subunit on Ca2+ sparks in permeabilized cardiomyocytes. We used wild-type (WT) and transgenic mice in which the highly phosphorylated site RyR2-S2808 has been ablated to investigate its involvement in RyR2 modulation. We further tested PP1 activity in other two mouse models (RyR2-S2030A and double knock-in RyR2-S2808/14A). In WT myocytes, where cytosolic Ca2+ was clamped at 45 nM, 2 U/ml of PP1 initially increased Ca2+ spark frequency (CaSpF) by 2-fold, followed by a second phase during which CaSpF returned to control. Due to the high CaSpF, spark- mediated leak was increased by PP1. This was accompanied by depletion of the sarcoplasmic reticulum (SR) Ca2+ stores, as determined by application of caffeine. Changes in Ca2+ release and SR Ca2+ load were prevented by 5 μM of okadaic acid, an inhibitor of PP1. S2808A mutant myocytes showed lower resting CaSpF compared to WT (1.68±0.11 vs 3.02±0.21 sparks/100 μm/s) and 2 U/ml of PP1 failed to generate changes in CaSpF as well as in SR Ca2+ load. A higher concentration of PP1 (10 U/ml) increased CaSpF 2.5-fold compared to control in WT, and 2.4-fold in S2808A cells, indicating a concentration-dependence. Similarly to S2808A cardiomyocytes, in S2808/14A myocytes 2 U/ml of PP1 did not change CaSpF, and 10 U/ml of the enzyme increased CaSpF 2.6-fold. In S2030A cardiomyocytes, 2 U/ml and 10 U/ml of PP1 increases CaSpF 1.4-fold and 1.7-fold respectively compared to control. Our results suggest that increased intracellular PP1 activity stimulates RyR2- mediated SR Ca2+ release and that de-phosphorylation of RyR2-S2808 and at least one not yet identified phosphorylation site may be important in RyR2 modulation.

P11. Role of Sox10-derived cells during cardiac regeneration in zebrafish

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Zebrafish has the capacity to regenerate the heart after an injury [1]. Regeneration after cryoinjury is preceded by massive fibrosis and scar formation around the injured area. The scar is removed and the cardiac regeneration is achieved by proliferation of pre-existing cardiomyocytes [2].
Sox10 is an established neural crest cell marker. Using the Sox10 ER^{T2} Cre line, Mongera *et al*. 2013 [3] identified a sox10-derived myocardial population in the developing zebrafish heart. In mammals, neural crest cells have been reported to contribute to the outflow tract and cardiac conductive system. Sox10-lineage tracing studies suggest that neural crest cells might also contribute to the myocardium in zebrafish.
Interestingly, we have found that embryonic sox10-derived cardiomyocytes are present in the adult zebrafish heart and we are studying the contribution of sox10-derived cardiomyocyte population in the adult heart and their contribution to cardiac regeneration upon ventricular cryoinjury.
Our results suggest that Sox10 expression is reactivated in response to cardiac injury in the adult zebrafish. Moreover, we have observed that cardiac regeneration is delayed when we use genetic ablation zebrafish model that conditionally remove sox10-derived cells.

We are currently elucidating the contribution of embryonic and adult sox10-derived cells to cardiac regeneration using gain and loss of function zebrafish models.

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2. González-Rosa, J. M. (2011) *Development* 138**:** 1663–1674
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P12. Novel strategies for the study of fibrosis regression during zebrafish heart regeneration

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The irreversible loss of millions of cardiomyocytes after a myocardial infarction is a major cause of morbidity and mortality worldwide. During the last decade, the mammalian adult heart has been shown to preserve a limited capacity of cellular proliferation and renewal after an injury. Zebrafish is a teleost that possess an endogenous high myocardial regenerative capacity by being able to eliminate the scar formed and partially restore the cardiac function after an insult. In this project, we are interested in studying the origin, heterogeneity and fate of fibroblasts subpopulations during the regenerative process. For that purpose, new zebrafish transgenic lines have been generated to perform double inducible lineage tracing of cardiac fibroblasts subpopulations during regeneration. This strategy will allow to genetically ablate these subpopulations and to evaluate their regenerative potential depending on their origin.

P13. Local crosstalk of inositol 1,4,5-trisphosphate receptor- and ryanodine receptor-dependent Ca^{2+} release in atrial myocytes

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In the heart, the dominant mechanism of intracellular Ca^{2+} release is Ca^{2+}-induced Ca^{2+} release (CICR) via sarcoplasmic reticulum (SR) Ca^{2+} release channels termed ryanodine receptors, RyRs). For cardiac myocytes Ca^{2+} release based on the intracellular second messenger inositol-1,4,5-trisphosphate (through $InsP_3R2$ activation) is not conclusively characterized and a functional crosstalk of $InsR_3Rs$ and RyRs on local scale is still enigmatic. However, $InsP_3$-induced Ca^{2+} release (IP3ICR) may play a significant role under pathophysiological conditions, e.g. atrial arrhythmogenicity. Compared to RyR based Ca^{2+} release events (Ca^{2+} "sparks") $InsR_3R$ Ca^{2+} release events (Ca^{2+} "puffs") are less frequent in cardiomyocytes and because of the signal-to-noise ratio are more difficult to identifiy. In addition, the lack of a fast fluorescent $InsP_3$ indicator and of a specific pharmacology for the $InsP_3R$ makes it challenging to examine RyRs-$InsP_3R$ interaction on subcellular scale.
In this study, elementary Ca^{2+} release events were examined in the presence/absence of IP3ICR / CICR in atrial myocytes. We hypothesized a functional crosstalk and/or cooperativity between both SR-Ca^{2+} release channels, e.g. IP3ICR may trigger or facilitate CICR via RyR activation and/or RyR sensitization or vice versa. Local calcium release events were identified and characterized in atrial myocytes acutely isolated from $InsP_3R2$ overexpressing mice by using rapid 2-D confocal imaging (150 Hz). $InsP_3R2$s were activated by ET-1 or with $InsP_3$ AM. Xestospongin C and 2-APB were used as antagonists of the $InsP_3R2$. Tetracaine was used as a RyR2 inhibitor. Alternatively, UV-flash photolysis approach of caged Ca^{2+} (DM-nitrophen, NP-EGTA) was applied. Conventional single event analysis often fails to separate / identify Ca^{2+} sparks and / or Ca^{2+} puffs by their elementary characteristics. The pixel-wise fitting algorithm of the data opens a way for analysis of individual events and to distinguish between Ca^{2+} sparks and Ca^{2+} puffs with higher precision. The quantitative analysis from 2-D data of individual selected Ca^{2+} events revealed Ca^{2+} puffs and Ca^{2+} sparks, respectively: Ca^{2+} sparks: 5 (1'000 $\mu m^2 s^{-1})^{-1}$, Ca^{2+} puffs: < 1 (1'000 $\mu m^2 s^{-1})^{-1}$.

However, Ca^{2+} puffs exist as discrete events in atrial myocytes with smaller amplitude ($\Delta F/F_0$=0.4), longer τ_{rise} and τ_{decay} (τ_{rise}=98 ms; τ_{decay}=130 ms) when compared to Ca^{2+} sparks. The FDHM found for Ca^{2+} puffs (367 ms) was significantly longer compared to Ca^{2+} sparks (64 ms).
In the presence of Xestospongin C remaining spontaneous local Ca^{2+} event activity was observed (Ca^{2+} sparks). Spontaneous local Ca^{2+} events are (nearly) completely absent in the presence of the RyR inhibitor tetracaine. Based on this pharmacological intervention and in combination with the new individual event analysis we classified these events as IP3ICR: Ca^{2+} puffs. This analysis suggests that in atrial myocytes $InsP_3$-induced Ca^{2+} release (Ca^{2+} puffs) may trigger individual Ca^{2+} sparks (CICR) - but there is also some evidence that initial Ca^{2+} spark activity (CICR, approached by UV-flash photolysis of caged Ca^{2+}) may trigger / boost IP3ICR given intracellular $InsP_3$ is present.

P14. Tolerance of cardiac mitochondria and vascular endothelium to ischemia and reperfusion

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Background: Organ shortage is a major issue in heart transplantation. In addition to conventional donation after brain death, donation after circulatory death (DCD) could significantly improve the number of cardiac grafts available for transplantation. However, DCD hearts undergo a period of warm ischemia and reperfusion (I/R).
Endothelial and early mitochondrial damage are key factors in cardiac I/R injury and their severity is determinant in vascular supply of the graft and cardiac myocyte survival, respectively.
The heart can withstand short periods of ischemia, but with increasing durations, the degree of post-ischemic cardiac dysfunction may strongly depend on the tolerance of both endothelium and myocardial mitochondria to I/R. Therefore, understanding the time-dependent effect of ischemia on the endothelium and cardiac mitochondrial integrity could help to improve the timing and choice of therapeutic targets for cardioprotection.
Objectives: We aim to characterize the tolerance of the cardiac endothelium and mitochondria to different durations of ischemia followed by reperfusion.
Methods: Experiments were performed with an isolated working rat heart model of DCD using modified Krebs-Henseleit buffer. Hearts were perfused in aerobic, working mode for 20 min, followed by either sham ischemia or periods of 21, 24, 27 or 30 min of warm, global ischemia and 60 min reperfusion. Hemodynamic parameters were continuously recorded. Endothelial function after 60 min reperfusion was evaluated by comparing endothelium-dependent (acetylcholine, Ach 10^{-7}M) and endothelium-independent (sodium nitroprusside, SNP $3x10^{-5}$) vasodilation. Mitochondrial integrity after 10 min reperfusion was monitored by measurements of oxygen consumption and cytochrome c (cyt c) release in coronary effluent.
Results:

	Hemodynamic function	**Mitochondrial integrity**	**Endothelial function**	
	LV work (%)	Cyt c release ($ng*min^{-1}*g^{-1}$)	Ach (% vasodilation)	SNP (% vasodilation)
No I	71±13	8.1	13±20	42±12
21 min I	69±3	ND	-17	44
24 min I	65±1	14.2 ± 5	-6±0.4	44±4.7
27 min I	60	29.8	-16	30
30 min I	41±2	36±1	-15±4	18±2

Table 1. Hemodynamic, mitochondrial and endothelial parameters
LV (left ventricular) work: (Developed Pressure*Heart Rate) expressed as a percentage of mean pre-ischemic value; Cyt c: cytochrome c release at 10 min reperfusion; Ach: Acetylcholine response expressed as percentage vasodilation at 60 min reperfusion; SNP: sodium nitroprusside response expressed as percentage vasodilation at 60 min reperfusion; ND: not determined.

Conclusion: Endothelial damage appears to precede the sharp decline in hemodynamic recovery and mitochondrial damage. Furthermore, our data support the concept that early cyt c release is a sensitive, early indicator of cardiac hemodynamic recovery. The characterization of endothelial and mitochondrial damage associated with cardiac I/R will help us to identify therapeutic approaches to optimize endothelial, mitochondrial and myocardial recovery.

P15. Opposite changes of Ca^{2+} wave threshold and fractional SR Ca^{2+} release during SERCA stimulation in cardiomyocytes

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In cardiac muscle, PKA-dependent phosphorylation of the RyRs is proposed to increase their Ca^{2+} sensitivity. This mechanism could be arrhythmogenic via facilitation of spontaneous Ca^{2+} waves. Surprisingly, the level of Ca^{2+} inside the SR ($[Ca^{2+}]_{SR}$) needed to initiate such waves has been reported to increase upon β-adrenergic stimulation, an observation which cannot be easily reconciled with elevated Ca^{2+} sensitivity of the RyRs. We tested the hypothesis that this change of Ca^{2+} wave threshold could occur indirectly, subsequent to SERCA disinhibition. Ca^{2+} currents and transients, or cytosolic and intra-SR Ca^{2+} waves were simultaneously recorded with confocal line-scans in intact and permeabilized mouse cardiomyocytes with rhod-2 and fluo-5-N, respectively. We analyzed changes of several Ca^{2+} signaling parameters during specific SERCA stimulation by ochratoxin A (OTA) and jasmone. SERCA stimulation resulted in a substantial increase of Ca^{2+} wave thresholds (30±5.1%) and reduced fractional Ca^{2+} release. Faster Ca^{2+} wave decay and SR refilling confirmed SERCA acceleration. In patch-clamped myocytes, a decrease of fractional Ca^{2+} release together with a slowing of Ca^{2+} current inactivation and reduced EC-coupling gain was observed. A faster Ca^{2+} transient decay corroborated the pharmacological SERCA stimulation. These results suggest that SERCA stimulation alone can elevate the intra-SR threshold for the generation of Ca^{2+} waves, independently of RyR phosphorylation. Unexpectedly, this occurs without noticeably increasing spontaneous and triggered fractional Ca^{2+} release. This phenomenon could result from an intra-SR mechanism limiting CICR. Supported by SNF.

P16. Calcium uncaging with visible light

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In cardiac myocytes a small amount of Ca2+ enters the cytoplasm upon depolarization and initiates Ca2+-induced Ca2+ release (CICR) from the sarcoplasmic reticulum, such release events can remain highly localized or initiate Ca2+ waves that propagate through the cell to trigger muscle contraction. Valuable optical method to control the concentration of intracellular calcium ions ([Ca2+]i) is uncaging. We have designed a nitroaromatic photochemical protecting group that absorbs visible light efficiently in the violet-blue range. The chromophore is a dinitro derivative of bisstyrylthiophene bearing two EGTA chelators (BIST-2EGTA). BIST-2EGTA has an absorption maximum at 440 nm, quantum yield of photolysis of 0.23, extinction coefficient at 440 nm of 66,000 M-1cm-1, two-photon cross section of 350 GM at 775 nm and binds Ca2+ with high affinity (Kd 84 nM at pH 7.2). Fluorescence imaging with a confocal microscope in point scan mode revealed that rhod-FF showed a rapid change in signal (<200 μs) when BIST- 2EGTA was photolyzed using two-photon (2P) excitation at 810 nm. Comparing to DM-nitrophen we found that the fluorescence signal from BIST- 2EGTA was about 13.7x larger when photolyzed under the same conditions. Efficiency of BIST-2EGTA to create changes in [Ca2+]i that are potentially useful for physiological studies was tested in whole-cell patchclamped cardiomyocytes loaded with BIST-2EGTA

and rhod-2 or X-rhod-5F. Short two-photon excitation at 810 nm produced localized Ca2+ releases that were considerably larger than those produced by BIST-2EGTA photolysis in caffeine-treated cells. Uncaging for longer periods triggered intracellular Ca2+ “mini-waves” or regular Ca2+ wave. Cells displayed normal excitation- contraction coupling, implying that BIST-2EGTA is nontoxic inside cells. One- photon uncaging with visible light also produced striking Ca2+ waves. BIST- 2EGTA has a set of properties that enables fast control of calcium inside living cells using visible light.

P17. S100A1 AAV-based gene transfer improves hemodynamic recovery from global ischemia-reperfusion injury in an isolated working rat heart model.

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Introduction: Inotropic S100A1 AAV-based gene therapy has shown rescue from post-ischemic heart failure in small and large animal models and is being developed towards clinical trials. Given its protective actions on cardiac metabolism and mitochondrial function in human failing cardiomyocytes, we hypothesized that S100A1 might beneficially influence recovery from ischemia-reperfusion injury.
Methods and Results: 4-5 fold cardiomyocyte-specific S100A1 overexpression (n=10) was achieved in male Lewis rats 4 weeks after systemic administration of an adeno-associated virus (AAV9-S100A1) while EGFP (AAV9-EGFP) served as control (n=8). Isolated hearts were perfused in working mode for 20 min, then subjected to 28 min global, no-flow, warm ischemia (37°C) and 60 min reperfusion. AAV9-S100A1 treated hearts showed improved hemodynamic function at 60 mins: Left ventricular (LV) developed pressure was 57% of baseline, 7% higher than control (50.9% (p=0.025), $dP/dt_{(max)}$ recovered to 61.6% of baseline, compared to 52.9% in controls (p=0.004) and LV work (HR x LVDP) was 7% higher in S100A1 overexpressing hearts (p=0.025). Consequently, lactate dehydrogenase (LDH) accumulation was 29% lower in the S100A1 group (p=0.018), indicating less tissue necrosis. In line with previously reported improved cardiac metabolism by S100A1, acetyl-CoA-carboxylase (ACC) phosphorylation was increased in AAV9-S100A1 treated hearts, indicating a potential benefit in S100A1 overexpressing myocardium.
Conclusions: We report for the first time, that AAV9-S100A1 treatment protects from cardiac reperfusion injury, potentially via diminished tissue necrosis and accelerated recovery of energy substrates. This new finding corroborates the therapeutic use of S100A1 and predicts protective potential in human hearts.

P18. Cardiac microRNA expression and release are regulated during early reperfusion after global ischemia in an isolated rat heart model of donation after circulatory death (DCD)

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Background: The lack of donor organs is a key limiting factor in heart transplantation as standard cardiac grafts, obtained through donation after brain death, cannot fulfill the rising demand. DCD hearts may be an option, although the inevitable period of warm ischemia followed by reperfusion (I/R) may induce permanent tissue damage that could prevent transplantation.
microRNA (miR), small, non-coding molecules, are crucial master regulators of key cellular processes in I/R injury, such as apoptosis, calcium overload and energy metabolism.
We investigated changes in cardiac miR expression during the first minutes of reperfusion following global ischemia, and their role as potential biomarkers of cardiac integrity in a rat model of DCD.
Methods: Hearts isolated from adult, male Wistar rats underwent baseline perfusion, followed by 27 min normothermic, global, no-flow ischemia and 10 min reperfusion. Left ventricular tissue (LV) was harvested from three experimental groups: “End Baseline” (EB) (n=14), “End Ischemia” (EI) (n=13), and “End Reperfusion” (ER) (n=11). At EB and ER, perfusate samples were harvested before and after passage through the coronary vasculature for 8 hearts. Levels of miR-1-3p, miR-15b-5p, miR-20a-5p, miR-21-5p,

miR-24-3p, miR-101b-3p, miR-133a-3p, miR-145-5p, miR-199a-5p, miR-223-3p, miR-320-3p, miR-494-3p and miR-499-5p were measured by RT-qPCR. Hemodynamic and biochemical parameters were also monitored.
Results: LV tissue expression was significantly reduced for miR-20a-5p at ER compared to EB and EI, and for miR-1-3p at ER compared to EB ($p<0.01$ for all). Expression of all other miR remained unchanged. Markedly increased levels of miR-1-3p, miR-145-5p and miR-499-5p were released by the heart after I/R injury vs pre-ischemia. Furthermore at ER, levels of several LV and circulating miR correlated with previously identified markers of post-ischemic hemodynamic recovery.
Conclusion: We show that changes in LV expression and cardiac release of miR occur within minutes of reperfusion following global ischemia. Our findings support an early, regulatory role for miR in I/R injury. Fluctuating miR levels may also provide evidence about post-ischemic hemodynamic recovery. Both LV and circulating miR represent potential therapeutic targets in cardiac I/R. Finally, the signature provided by the combination of hemodynamic, biochemical and miR measurements may be used to predict post-ischemic recovery and evaluate DCD cardiac graft suitability.

P19. Cardiac regeneration in zebrafish: a role for LOX

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In recent years the zebrafish was established as a cardiac regeneration model and has been widely used. Although different types of injuries have been developed (resection, cryoinjury and genetic ablation) our studies have all been conducted using the cryoinjury model. This is the model that more closely resembles the events occurring after myocardial infarction in humans, since it induces massive cell death and the deposition of fibrotic tissue. However, in zebrafish this fibrotic deposition is transient. The accumulated fibroblasts as well as the extra-cellular matrix that form a scar are eliminated within a few months of cryoinjury. Thus, fibrotic repair and regeneration are not incompatible processes.

We proposed to understand the elimination of the fibrotic tissue by looking into the role of the extracellular matrix in this process. We have found that the lysyl oxidase gene family, which facilitates collagen and elastin cross-linking, was up-regulated immediately upon cryoinjury. We have postulated that chemical inhibition of these enzymes (using 3-Aminopropionitrile) would further improve regeneration, considering that a lack of collagen cross-linking would prevent the maturation of these fibers, hence preventing the formation of mature and hard scar tissue and helping the elimination of the formed fibrotic clot. However, we have observed that blocking of lox enzymes immediately after a cryoinjury has a long lasting effect, which results in a delayed formation of new myocardium, impeding the normal regenerative process.
Hence, we believe that LOX enzymes play an essential role in ventricular regeneration upon injury.

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P20. Epigenetically mediated exaggerated vascular responsiveness to Angiotensin II in ART mice

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Introduction: Environmental influences acting early in life predispose to premature cardiovascular diseases (CVD). Assisted reproductive technologies (ART) involve the manipulation of early embryos at a

time when they are particularly vulnerable to external disturbances. In line with this concept, we recently showed that ART induces endothelial dysfunction, premature vascular ageing and arterial hypertension in humans and mice. These problems are related, at least in part to epigenetic modifications of the endothelial nitric oxide synthase (eNOS) gene resulting in decreased vascular nitric oxide (NO) bioavailability. However, it is unknown if others mechanisms also contribute to ART-induced alteration of the cardiovascular phenotype. In rodents, restrictive diet during pregnancy or prenatal nicotine exposition induce arterial hypertension in the offspring by increasing the vascular angiotensin II (ANG II) sensitivity. We speculated that a similar mechanism could be involved in ART-induced arterial hypertension.
Method: To test this hypothesis, we assessed ANG II sensitivity in ring preparations of the distal aorta of ART and control mice by measuring the vasoconstrictor responsiveness to stepwise increasing doses of ANG II in the presence of an eNOS inhibitor (L-NMA). We also quantified ANG II receptors (AGTR) type 1 and 2 expression (Western Blot) and measured the methylation state of the AGTR gene promoters (bisulfite sequencing) in the aorta. Moreover, we assessed endothelial function (mesenteric-artery responsiveness to increasing doses of acetylcholine) and in vivo arterial blood pressure (using a carotid catheter).
Results: As expected, ART mice displayed marked mesenteric-artery endothelial dysfunction (P=.03, vs. control) in vitro and arterial hypertension in vivo (121.8±7.3 vs. 114.6±4.5 mmHg, P=.02, vs. control). Most importantly, the vasoconstrictor response to ANG II, independently of endothelial function, was significantly greater in ART than in control mice (P<.01, vs. control). In line with these findings, in the aorta the AGTR 1/2 ratio of protein expression was significantly increased (P<.008, vs. control) and the promoter of the AGTR 1b gene was hypomethylated in ART compared with control mice.
Conclusion: Here, we show for the first time that ART increases the angiotensin II sensitivity in the aorta. This increased sensitivity is due to an epigenetically mediated imbalance between the expression of the vasoconstrictive (AGTR type 1) and the vasodilatator (AGTR type 2) receptor. Hence, we identified a new mechanism, independent of the NO pathway, that appears to be involved in the pathogenesis of ART-induced premature vascular ageing and arterial hypertension in mice. We speculate that this mechanism also contributes to ART-induced premature vascular ageing and arterial hypertension in humans.

P21. TRPM4 variants in childhood atrio-ventricular block.

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TRPM4, a non-selective calcium-activated cation channel has been known for its decisive role in electrical activity of heart. Dominantly inherited mutations in *TRPM4* gene are associated with cardiac conduction diseases and Brugada syndrome. Despite many new findings regarding TRPM4 role in cardiac conduction, the mechanisms underlying TRPM4-dependent conduction disorders are not fully understood. Here in this study we characterized TRPM4 genetic variants found in patients with congenital or childhood atrio-ventricular block (AVB). Five rare *TRPM4* genetic variants (D198G, A432T, G582S, T677I, and V921I) were identified from ninety one congenital or childhood AVB patients. These variants were further subject to biochemical and functional studies. The G582S variant was also found to be isolated with A432T variants in two patients belonging to two unrelated families, so we included a double variant A432T/G582S in our study. Functional and expression studies in a heterologous expression system

identified two of these variants A432T and A432T/ G582S with loss- and variant G582S with gain-of-function. Unlike previous reports, we did not find any evidence to confirm a direct or indirect role of SUMOylation in the gain-of-function *TRPM4* variants. However, the loss-of-function TRPM4 variants could be likely due to protein misfolding and retention in the endoplasmic reticulum, as a reduced incubation temperature was able to partially rescue their expression and function. However, the loss-of-function is not explained *via* an increase of ubiquitylation of TRPM4 protein. Our present study further supports the role of this channel in cardiac conduction, particularly in atrio-ventricular conduction. It can be, therefore, proposed that this gene be added to the list of candidates to be screened in patients with conduction disease and BrS.

P22. Generation, validation and characterization of an adult-inducible cardiac-specific SIRT1 knock-out mouse model

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Objective: The role of energy deficit in the development and progression of heart failure is well established and Sirtuin1 (Sirt1), as cellular sensor, is crucially involved. To unravel the in vivo function of Sirt1 in the heart, several transgenic mouse models of Sirt1 have already been studied. However, none of these have addressed the precise role of Sirt1 on the heart function in adulthood. To address this, we have generated the first adult cardiac-specific and inducible Sirt1 KO mouse model (Sirt1 CiKO).
Results: Sirt1 CiKO mouse strain was generated by crossing mice carrying loxP sites flanking exon 4 $Sirt1^{\Delta Ex4}$ (gift of Dr Sinclair, USA) with tamoxifen-inducible Cre recombinase under the control of the alpha-myosin heavy chain promoter mice α-MHC-$MCM^{wt/cre}$ (courtesy of Dr Merisckay, France). Deletion of Sirt1 Exon4 was induced by two consecutives days of 40 mg/kg tamoxifen injection at an adult state (8 weeks old). Four weeks after tamoxifen injection (a.t.i), a partial loss of 60% in Sirt1 protein in heart tissue was reported in the CiKO model. The follow-up exploration of cardiac function during 4 months showed that from 11 weeks (a.t.i) CiKO mice presented an altered systolic function, which was maintained until 4 and 9 months. At 4 months (a.t.i), mitochondrial respiration in CiKO mice tended to be lower than in control mice although without significance. Interestingly, a decompensation in respiration capacity was found in older Sirt1 CiKO animals (11 monts a.t.i), accompanied by a lower citrate synthase activity. Importantly, mitochondrial H_2O_2 production was increased by 30% in the transgenic model at both ages. While pressure overload applied during 4 weeks led to a compensated hypertrophy stage in control mice, a higher degree of hypertrophy associated with decreased ejection fraction (by 30%) were found in CiKO mice.
Conclusions and perspectives: Our study conducted with the novel adult cardiac-specific and inducible Sirt1 KO mouse model shows a diminution of cardiac ejection fraction in both normal and failing mouse hearts, suggesting an initial systolic dysfunction, which could be related to a higher oxidative stress in the KO model. Future analyses are needed to understand the deleterious effect of Sirt1 deletion in the adult heart.

P23. Remake of Cardiac Safety Assessment – HERG Discontinued?

Thomet U et al.

During the past decade, the assessment of proarrhythmic toxicity of new drug applications has attracted significant attention from regularity authorities and pharmaceutical industries. Since 2005, when the ICH S7B guideline has been issued by the International Conference on Harmonization (ICH), the establishment of cardiac safety pharmacology requires solid preclinical investigation. Although studies in line with the current safety paradigm may correctly predict the clinical outcome, a number of false negatives and false

positives have been reported. The purpose of the presentation is to review the limitations of the recommended preclinical assays and to announce a newly established multimode approach for fast in vitro safety profiling. Around 20% of the ICH S7B studies are in discordance with the clinical findings. Rather than addressing proarrhythmia as direct endpoint of clinical concern, the prolongation of QT interval is considered. Second, the block of the hERG channel is not always sufficient to predict cardiac safety flags. Therefore, a comprehensive validation of various cardiac ion channel effects along with the analysis of human cardiomyocytes can more accurately assess the proarrhythmic potential of a drug candidate. In vitro patch-clamp data from recombinant cell models expressing human sodium, calcium and potassium channels (hNav1.5, hCav1.2, hERG, hKvLQT1/minK, Kir2.1) are discussed. Action potential analysis in stem cell derived cardiomyocytes revealed repolarization and proarrhythmic drug effects. Furthermore, in depth analysis of late sodium currents, state and use dependence of channel block, hNCX1 transporter modulation and hTRPM4 interaction assays assisted the thorough evaluation of the cardiac safety battery. In summary, due to the documented disadvantages with the ICH S7B preclinical models, it is proposed to accomplish multiple cardiac ion channel studies when assessing the proarrhythmic toxicity of new promising molecules. Application of a CIPA (Comprehensive In Vitro Proarrhythmia Assay) driven concept during preclinical stages shall help inject more drugs with potentially significant health benefits into market earlier.

P24. Atrophic remodeling limits contractile function of long-term unloaded hearts assessed via *ex vivo* whole heart perfusion

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Objective: In a subset of patients, substantial recovery of ventricular function after mechanical unloading by left ventricular assist devices (LVADs) can be observed. However, myocardial unloading via LVAD confers not only beneficial remodeling of the failing myocardium, but also detrimental loss of myocardial mass. The consequences of mechanically unloading the heart can be mimicked in a small animal model of heterotopic heart transplantation (HTX) in isogenic rats. The complex response to mechanical unloading necessitates reproducible functional assessment of the remodeled heart on a cellular and organ level.
Methods: Normal rat hearts were mechanically unloaded by heterotopic transplantation for 30 days. Functional capacity of unloaded hearts (UN, n=4) and age-matched control hearts (CTR, n=5) was assessed in an ex vivo working heart perfusion system. We analyzed hemodynamic parameters (developed pressure (dP), contractility and relaxation (dP/dt max/min), end-diastolic pressure (EDP), heart rate (HR), cardiac output(CO), LV work (dP×HR)) using pressure catheters, as well as oxygen consumption and lactate production. Inotropic response to increasing catecholamine doses (isoproterenol) was tested.
Results: After 30 days of unloading via HTX, UN hearts showed significant atrophy with a diminution of heart weight by 46%. Our data show that contractile function and cardiac output is impaired at baseline in unloaded hearts: dP was 131±22mmHg in CTR vs 84±16mmHg in UN hearts, CO was 47 ml/min in CTR and 15 ml/min in UN group. In addition, contractile function was limited at baseline (dP/dt max of 4353±377 vs 2594±443 in UN) as well as under β-adrenergic stimulation with isoproterenol. Inotropic response in the unloaded hearts showed an increase by maximally 87% regarding dP/dt max in contrast to 166% in CTR hearts.
Conclusion: This is the first report of ex vivo working heart perfusion in unloaded rat hearts. This model was successfully used to evaluate unloaded heart function under loaded conditions and our data can add important information on the capability of the atrophic heart with implications on potential recovery of failing unloaded hearts. Diminished function of unloaded hearts at baseline and under β-adrenergic stimulation implies that unloading-induced atrophic remodeling as such has detrimental effects on the capability of the myocardium that have to be addressed in view of a potential use of LVAD as a therapeutic option in heart failure patients.