

**BELGIAN SOCIETY OF
PHYSIOLOGY AND PHARMACOLOGY**

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting
Friday, April 29th 2022

PROGRAMME

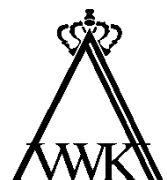
Venue

**Palace of the Academies
Royal Academy of Medicine of Belgium
“Rubens room”
Rue Ducale / Hertogstraat 1
1000 Brussels**

Local host

**Prof. Dr. Julien Hanson
Laboratory of Molecular Biology – GIGA Institute
ULiège
Belgium**

**with support of the
Royal Flemish Academy of Belgium for Science and the Arts**



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Spring Meeting
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Venue

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“Rubens room”
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1000 Brussels

09.15-10.00 Welcome with coffee and tea

Keynote lecture

10.00-11.00 **Beyond the consensus G protein coupling profile of GPCRs: towards physiology and system bias**
Ralf Jockers (Institut Cochin, INSERM, Paris)

Oral communications (morning session)

11.00-11.15 **Role of macro-molecules in activating human Mas-related G protein-coupled receptor D: allosteric/orthosteric ligand or mechanosensitive?**
Rohit Arora, Joni Heymans, Nikola Papovic, Kenny M. Van Theemsche, Alain J Labro (UGhent & UAntwerp)

11.15-11.30 **A non-canonical effect of BAPTA inhibits glycolysis, resulting in cell death in Mcl-1-dependent cancers**
Sneyers F., Kerkhofs M., Welkenhuyzen K., Ghesquière B., Dewerchin, M., Bootman M.D., Bultynck G (KULeuven)

11.30-11.45 **Changes in irisin (FNDC5) and myostatin expression in doxorubicin-treated C2C12 myotubes**
Van Asbroeck Birgit, Dombrecht Dorien, Van Craenenbroeck Emeline, Guns Pieter-Jan, Van Breda Eric (UAntwerp)

11.45-12.00 **Early detection of chemotherapy induced cardiotoxicity**
Dustin Krüger, Matthias Bosman, Constantijn Franssen, Pieter-Jan Guns (UAntwerp)

12.00-12.15 **Glucose inhibits glucagon secretion of mice with α -cell-specific deletion of KATP channels**
Manjitha Parambath, Bao-Khanh Lai, Heeyoung Chae, Eva Gattineau, Susumu Seino, Patrick Gilon (UCL & Kobe Univ Japan)

12.15-12.30 **TRPM3 – novel target to alleviate chemotherapy-induced peripheral neuropathic pain (CIPNP)**
Vincenzo Davide Aloï, Silvia Pinto, Rita van Bree, Katrien Luyten, Thomas Voets, Joris Vriens (KULeuven)

12.15 – 13.00 Lunch and Posters

Posters:

- **Neuromorphic circuits for undergraduate neurophysiology courses**
Sébastien Szczepanski, Maëlle Limpens, Alexandre Legrand, Anne-Emilie Declèves, Alexandra Tassin (UMons)
- **Adiponectin pathway in a murine model of disuse muscle atrophy: an in vivo study**
Shlyonsky V, Dupuis F, de Prelle B, Gall D (ULB)

Oral communications (afternoon session)

13.15-13.30 **The important role of PAR2 in early implantation processes**
Martina Ciprietti, Joris Vriens (KULeuven)

13.30-13.45 **TRPV2 is involved in trophoblast branching of the mouse placenta**
Katrien De Clercq, V. Perez-Garcia, J. Lopez-Tello, E. Van Den Broek, T. Voets, A. Sferruzzi-Perri, J. Vriens (KULeuven & UCambridge UK)

13.45-14.00 **Glycemic control in TRPM4 knockout mice during pregnancy**
Caroline Wuyts, Koenraad Philippaert, Silvia Pinto, Caroline Simoens, Rudi Vennekens (KULeuven)

14.00-14.15 **Studying endometrial dysfunction in PCOS with a prenatally androgenized mouse model**
Luyckx L, Virtanen N, Arffman R, Prunskaitė-Hyryläinen R, Piltonen T, Vriens J (KULeuven)

14.15-14.30 **Effect of a rare mutation of the Nav1.4 channel, L1436P, on its biophysical properties**
L. Dubois, Y. Sun, K. Jacquerie, B. Lakaye, FC Wang, V. Seutin (ULiège)

14.30-14.45 **Cardiomyocyte differentiation from iPS cells is delayed following knockout of Bcl-2**
Tim Vervliet (KULeuven)

14.45 – 15.15 Coffee – Tea

Closing lecture

15.15-16.15 **TRPM3 – a hot channel on steroids**
Thomas Voets (KULeuven)

ORAL PRESENTATION 1

Role of macro-molecules in activating human Mas-related G protein-coupled receptor D: allosteric/orthosteric ligand or mechano-sensitive?

Rohit Arora(1), Joni Heymans(2), Nikola Papovic(2), Kenny M. Van Theemsche(1,2), Alain J Labro(1,2)

(1) Laboratory for Molecular, Cellular and Network Excitability, Department of Biomedical Sciences, University of Antwerp, 2610 Wilrijk, Belgium (2) Department of Basic and Applied Medical Sciences, Ghent University, 9000 Ghent, Belgium

INTRODUCTION | Mas-related G protein-coupled receptors (MRGPRs) are known to mediate itch, pain, and pseudo-allergic reactions. A member of MRGPRs family, MRGPRD has been reported to mediate the release of inflammatory cytokines like TNF- α , IL-1 β and IL-6. Since MRGPRD has emerged as a pivotal member in the regulation of the renin-angiotensin system, there is growing evidence of an important role in the cardiovascular system. In our previous study, it was noticed that MRGPRD displays a high basal activity in presence of fetal bovine serum in the culture medium. Since G protein-coupled receptors are known to be activated and modulated by sterols, we evaluated the effect of cholesterol and bile acids on MRGPRD activity.

METHODS | HeLa cells stably expressing human MRGPRD were stimulated with cholesterol, bile acids or methyl- β -cyclodextrin (MBCD) for 8 h. After 8 h, supernatant was collected, and IL-6 estimation was performed using ELISA.

RESULTS | The IL-6 release was observed from MRGPRD-expressing HeLa cells treated with cholesterol and bile acids, indicating the activation of MRGPRD. However, methyl- β -cyclodextrin (MBCD), which extracts sterols from the membrane also induced the IL-6 release.

CONCLUSION | Considering both addition and depletion of sterols caused MRGPRD activation, these findings are indicative of mechano-sensitivity of MRGPRD. Therefore cholesterol, bile acids and MBCD mediated activation of MRGPRD needs further investigation to elucidate if it is an allosteric/orthosteric or mechano-sensitive effect.

ORAL PRESENTATION 2

A non-canonical effect of BAPTA inhibits glycolysis, resulting in cell death in Mcl-1-dependent cancers

Sneyers F., Kerkhofs M., Welkenhuyzen K., Ghesquière B., Dewerchin, M., Bootman M.D., Bultynck G

KULeuven

A hallmark of B-cell malignancies such as diffuse large B-cell lymphoma (DLBCL) is anti-apoptotic Bcl-2 overexpression, enabling cancer cells to escape apoptosis by neutralizing pro-apoptotic proteins. Previous work in the DLBCL model OCI-LY-1 indicated that the intracellular Ca^{2+} chelator BAPTA (BAPTAi) synergistically enhanced apoptosis elicited by the BH3-mimetic Bcl-2 antagonist venetoclax. Therefore, we set out to understand the mechanisms underlying the synergism between venetoclax and BAPTAi. Here, we demonstrate that BAPTAi by itself could induce apoptosis in OCI-LY-1. We found that BAPTAi provoked a complete and rapid decline in Mcl-1-protein levels through inhibition of translation. Remarkably, overexpression of a non-degradable Mcl-1 variant resulted in the rescue of BAPTAi-induced cell death, indicating that the apoptotic effect of BAPTAi was caused by loss of Mcl-1. Lastly, we found that BAPTAi rapidly suppressed glycolytic activity by inhibiting PFKFB3, a master regulator of the rate-limiting step of glycolysis, lying at the heart of translation inhibition and Mcl-1 downregulation. Surprisingly, all aforementioned effects of BAPTAi could be phenocopied by a BAPTAi variant compromised in buffering intracellular Ca^{2+} . Moreover, EGTAi, a structurally different Ca^{2+} chelator with similar affinity for Ca^{2+} as BAPTAi, was much less effective in lowering Mcl-1-protein levels and did not provoke cell death. Thus, our results reveal an unconventional, “ Ca^{2+} -independent” mechanism by which BAPTAi limits survival of DLBCL, exploiting their dependence towards Mcl-1.

ORAL PRESENTATION 3

Changes in irisin (FNDC5) and myostatin expression in doxorubicin-treated C2C12 myotubes

Van Asbroeck Birgit, Dombrecht Dorien, Van Craenenbroeck Emeline, Guns Pieter-Jan, Van Breda Eric

Department of Rehabilitation Sciences & Physiotherapy, Research group MOVANT, University of Antwerp

INTRODUCTION | Doxorubicin (DOX) is a widely used chemotherapeutic agent in certain types of cancer. It causes, however, a toxic effect on the heart and skeletal muscle. In skeletal muscle, DOX leads to muscle atrophy (wasting). As an important secretory organ, skeletal muscle secrete myokines in response to contraction. Hence, myokines play an important role in the regulation of skeletal muscle mass and metabolism. However, the role of myokines in DOX-induced skeletal muscle toxicity is unknown.

METHODS | Fully differentiated C2C12 myotubes were treated with or without DOX (0.2 μ M, 1 μ M or control). Cell viability was evaluated 24h, 48h and 72h after exposure. Based on the cell viability findings, proteins were extracted from myotubes 48h following exposure. The expression of decorin, irisin (FNDC5) and myostatin were evaluated by western blotting and quantified using Empiria Studio.

RESULTS | DOX (1 μ M) treatment of cultured myotubes significantly decreased the expression of irisin (FNDC5) and tended to increase myostatin expression. Decorin levels remained unchanged. Additionally, several markers of protein synthesis (AKT and Eef2) and protein degradation (Atrogin-1 and AMPK) showed deregulation.

CONCLUSION | Our data show for the first time that DOX causes a downregulation of irisin (FNDC5) in DOX-treated C2C12 myotubes. Our findings suggest that the altered regulation of irisin (FNDC5) and myostatin are likely to contribute to the development of DOX-induced skeletal muscle wasting. Future in vivo studies will help to unravel the role of myokines in DOX-induced skeletal muscle wasting.

Early detection of chemotherapy induced cardiotoxicity

Dustin Krüger, Matthias Bosman, Constantijn Franssen, Pieter-Jan Guns

University of Antwerp

INTRODUCTION | Doxorubicin (DOX) is one of the most used and effective chemotherapeutics, which is also known to be potentially cardiotoxic. Besides i.a. apoptosis of cardiomyocytes, and left ventricular (LV) dysfunction, DOX treatment causes eventually congestive heart failure (HF). While survival rates of cancer patients are improving, cardiovascular adverse effects of chemotherapeutics have become a growing concern. Thus, cancer patients treated with DOX may become tomorrow's HF patients. In the current project we perform translational research aiming to thrive novel strategies to identify patients at risk of developing cardiotoxicity upon DOX treatment. A possible first step in the development of cardiotoxicity impaired diastolic function may serve as an early marker for patient stratification and follow-up.

METHODS | C57BL6 mice (n=6-12) were treated with DOX (4 mg/kg, 1 weekly i.p. injection) for 2 and 6 weeks while cardiovascular function was investigated longitudinally with ultrasound imaging and terminal with invasive pressure-volume (PV) loops. Furthermore, samples were collected for molecular and histological analysis.

RESULTS | LV ejection fraction (EF) was decreased during 6weeks of DOX treatment. Interestingly, abdominal PWV was increased at 2 weeks (vehicle: 3.124 ± 0.097 m/s; DOX: 4.506 ± 0.286 m/s) pointing towards increased arterial stiffness, likely reflecting endothelial dysfunction. PV-loop analysis showed an increase in dP/dt min and Tau (vehicle: 5.969 ± 0.250 s; DOX: 8.384 ± 0.751 s), indicating prolonged relaxation time.

CONCLUSION | Overall, these data support the concept that endothelial dysfunction and impaired diastolic function occur early in the pathogenesis of DOX cardiotoxicity.

Glucose inhibits glucagon secretion of mice with α -cell-specific deletion of KATP channels

Manjitha Parambath¹, Bao-Khanh Lai¹, Heeyoung Chae¹, Eva Gattineau¹, Susumu Seino², and Patrick Gilon¹

¹Université Catholique de Louvain, Institut de Recherche Expérimentale et Clinique, Pôle d'Endocrinologie, Diabète et Nutrition, Brussels, Belgium; ²Division of Molecular and Metabolic Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

The islets of Langerhans contain α -, β - and δ -cells secreting glucagon, insulin, and somatostatin (SST), respectively. Glycemia is controlled by insulin (hypoglycemic) and glucagon (hyperglycemic). The secretion of both hormones is inhibited by SST. α -, β - and δ -cells express KATP channels. Glucose stimulates insulin and SST secretion by closing β - and δ -cell KATP channels. By contrast, it inhibits glucagon release by largely unknown mechanisms. In particular, the role of α -cell KATP channels in the glucagonostatic effect of glucose is debated. To investigate it, we generated mice lacking KATP channels in α -cells (α -KATPKO) and expressing or not SST (SSTKO). To evaluate the role of $[Ca^{2+}]_c$ changes on glucagon release, we crossed these mice with a reporter mouse expressing the fluorescent Ca^{2+} probe, GCaMP6f, specifically in α -cells. We observed that changing the glucose concentration from 1 to 7mM, inhibited glucagon release of α -KATPKO and control islets expressing or not SST, suggesting, glucose can control glucagon secretion independently of α -cell KATP channels and SST. The addition of the KATP channel opener, diazoxide, in 1 mM glucose suppressed glucagon release of control islets. Interestingly, it stimulated glucagon secretion of α -KATPKO islets but it was without effect in α -KATPKO/SSTKO islets. These changes in glucagon secretion mirrored modest changes in α -cell $[Ca^{2+}]_c$. Overall, these data suggest that glucose controls glucagon release independently of α -cell KATP channels. However, pharmacological opening of α -cell KATP channels suppresses glucagon release whereas opening of δ -cell KATP channels stimulates glucagon secretion by alleviating the glucagonostatic effect of SST released by δ -cells.

TRPM3 – novel target to alleviate chemotherapy-induced peripheral neuropathic pain (CIPNP)

Vincenzo Davide Aloï, Silvia Pinto, Rita van Bree, Katrien Luyten, Thomas Voets, Joris Vriens

Laboratory of Endometrium, Endometriosis and Reproductive Medicine, Department of Development and Regeneration, KU Leuven, Leuven, Belgium. Laboratory of Ion Channel Research, VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium. Department of Molecular Medicine, KU Leuven, Leuven

Chemotherapy-induced peripheral neuropathic pain (CIPNP) is an adverse effect observed in up to 80% of cancer patients upon treatment with cytostatic drugs including paclitaxel and oxaliplatin. Patients complaints of CIPNP can be so severe that it limits dose and choice of chemotherapy, and has a significant negative consequences on the quality of life of survivors. Current treatments for CIPNP are limited and unsatisfactory. TRPM3 is an Ca^{2+} -permeable ion channel functionally expressed in peripheral sensory neurons involved in the detection of thermal stimuli. Here, we focus on the possible involvement of TRPM3 in acute oxaliplatin induced mechanical and cold hypersensitivity. In vitro calcium microfluorimetry and whole cell patch clamp experiments showed that TRPM3 is functionally upregulated in both heterologous and homologues expression systems after acute (24h) oxaliplatin treatment, while direct application of oxaliplatin was without effect. In vivo behavioral studies using an acute oxaliplatin model for CIPNP showed the development of cold and mechano-hypersensitivity in control mice, which was lacking in TRPM3 deficient mice. Moreover, intraperitoneal injection of a TRPM3 antagonist, isosakuranetin, effectively reduced the oxaliplatin induced pain behavior in response to cold and mechanical stimulation in mice with an acute form of oxaliplatin induced peripheral neuropathy (OXAIPN). In addition, the levels of pERK, a marker for neuronal activation after noxious stimulation, were significantly reduced in DRG neurons derived from TRPM3 deficient mice compared to control after oxaliplatin administration. These findings suggest TRPM3 as an interesting molecular target to reduce oxaliplatin induced peripheral neuropathy.

The important role of PAR2 in early implantation processes

Martina Ciprietti, Joris Vriens

Laboratory of Endometrium, Endometriosis and Reproductive Medicine (LEERM)
Department of Development and Regeneration, KULeuven

The embryo implantation process is a complex phenomenon characterized by the presence of an implantation-competent embryo, receptive maternal endometrium and the intricate crosstalk between both. Successful implantation is contingent on the optimal unity between these factors. Proteases have been described as blastocyst-secreted proteins involved in the early implantation events. They stimulate intracellular calcium signaling pathways in endometrial epithelial cells, leading to optimal maternal receptivity and successful embryo implantation. Unfortunately, the exact molecular players underlying protease-induced calcium signaling, and the subsequent downstream pathways remain elusive. This research evaluates the effect of long-term stimulation by the serine protease trypsin in endometrial epithelial cells and aims at investigating the molecular mechanisms at play. Long-term application of trypsin induced calcium waves in EEC of mouse and human, dependent on phospholipase C, inositol 1,4,5-trisphosphate and store-operated calcium entry pathways. Moreover, protease-activated receptor-2 (PAR-2) was identified as the molecular entity initiating protease-induced calcium responses.

TRPV2 is involved in trophoblast branching of the mouse placenta

Katrien De Clercq (1,2), V. Perez-Garcia (3,4), J. Lopez-Tello (4), E. Van Den Broek (2), T. Voets (2), A. Sferruzzi-Perri (4), J. Vriens (1)

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INTRODUCTION | The placenta mediates nutrient supply to sustain fetal growth during pregnancy. A large exchange area is created by extensive branching morphogenesis of the trophoblasts. Pivotal to this labyrinth formation is the differentiation and branching of Syncytiumtrophoblast layer II (SynTII). However, little is known regarding the underlying pathways that underpin placentation. Calcium signals are well suited to translate signals from the environment into a cellular response. Previously, we identified a crucial role of the calcium-permeable channel TRPV2 in placental development as *Trpv2*^{-/-} mice display intra uterine growth restriction (IUGR) and lethality. Here, we investigated the role of TRPV2 in placental development and trophoblast branching.

RESULTS | Global genetic ablation of *Trpv2* results in IUGR and embryonic lethality at E18.5. Deletion of *Trpv2* in the embryo proper or in the junctional zone of the placenta was compatible with normal development, suggesting that labyrinthine *Trpv2* is indispensable for normal fetal development. In the labyrinth, *Trpv2* is highly expressed in SynTII trophoblast cells. Moreover, the labyrinth morphology of *Trpv2*^{-/-} placentas was severely disturbed, with regions where no that are void of both SynTII cells and fetal vessels. RNAsequencing of wildtype and *Trpv2*^{-/-} placentas revealed that branching morphogenesis and angiogenesis pathways were affected. Interestingly, Crispr/Cas9-mediated deletion of *Trpv2* in Trophoblast Stem Cells resulted in impaired differentiation of the SynTII labyrinth lineage.

CONCLUSION | Our observations suggest that loss of TRPV2 in mice disrupts the differentiation of SynTII trophoblasts, which likely contributes to defects in labyrinth morphogenesis, nutrient supply, and thus compromised fetal growth.

Glycemic control in TRPM4 knockout mice during pregnancy

Caroline Wuyts, Koenraad Philippaert, Silvia Pinto, Caroline Simoens and Rudi Vennekens

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INTRODUCTION | During pregnancy, the glucose homeostasis of the mother adapts to accommodate the needs of a growing fetus. These adaptations include increased insulin secretion from the pancreatic beta-cell. Electrophysiological changes in the beta-cell during pregnancy, that could explain this adaptation, have not yet been identified. The non-selective Transient Receptor Potential Melastatin 4 (TRPM4) cation channel, expressed in beta-cells, may influence the insulin release. In other organs, TRPM4 ion channel expression fluctuates depending on the relative concentration of estrogen and progesterone. These findings suggest a role of TRPM4 in glycemic control, which can be influenced by female reproductive hormones during pregnancy.

METHODS | Using in vivo telemetric continuous glucose monitoring throughout the full pregnancy of wildtype (WT) and TRPM4 KO mice allows to analyze glycemia during oral glucose tolerance tests (OGTTs) and spontaneous feeding behavior, and to determine glycemic variability.

RESULTS | TRPM4 KO mice are hyperglycemic compared to WT mice, mainly before and during the first half of pregnancy. The maximum glucose levels reached during the OGTTs and during spontaneous glucose excursions were larger for the TRPM4 KO mice compared to the WT mice. Glycemic variability, a measure for sudden changes and fluctuations in the blood glucose concentration, did not show substantial differences between both genotypes.

CONCLUSION | To conclude, we could show that TRPM4 plays an active role in glycemic control, before and during pregnancy.

Studying endometrial dysfunction in PCOS with a prenatally androgenized mouse model

Luyckx L, Virtanen N, Arffman R, Prunskaitė-Hyyryläinen R, Piltonen T, Vriens J

KU Leuven

INTRODUCTION. Polycystic ovary syndrome (PCOS) is a fertility disorder in women characterized by reproductive defects including irregular menstruation and pregnancy complications, but also by metabolic derangements such as obesity, insulin resistance and cardiovascular diseases. It is hypothesized that PCOS originates from prenatal androgenization (PNA), but the effects of PNA on endometrial function are currently understudied. Therefore, this project aimed to study endometrial dysfunction in a PNA PCOS-like mouse model.

METHODS. To induce PCOS, pregnant female mice received 250 µg dihydrotestosterone during E16.5-E18.5. The pups received a special diet to induce obesity. The anogenital distance and estrus cyclicity were assessed. After ovariectomy, the mice received estrogen and progesterone injections to mimic the natural cycle followed by euthanasia during the window of implantation. Alternatively, in addition to the above protocol, 25 µl sesame oil was injected into the uterine lumen to induce the decidualization reaction followed by uteri collection for analysis via qPCR and IHC.

RESULTS. PNA mice showed increased anogenital distance and disrupted estrus cycles. PNA mice had reduced expression of typical receptivity genes including *Ccnd1* and *2*, *Itgb3* and *Muc1* during the window of implantation compared to controls. Additionally, PNA mice had an impaired decidualization response as demonstrated from the reduced weights of the injected uterus horns.

CONCLUSION. This data shows that PNA affects reproductive function by disrupting estrus cyclicity and by directly affecting endometrial function during the window of implantation and during decidualization. This research can contribute to the development of effective fertility treatments for women with PCOS.

Effect of a rare mutation of the Nav1.4 channel, L1436P, on its biophysical properties

L. Dubois, Y. Sun, K. Jacquerie, B. Lakaye, FC Wang* and V.Seutin

GIGA Research, University of Liège and *department of Physical Medicine, University Hospital of Liège

INTRODUCTION The muscle Nav channel, Nav1.4, is crucially involved in skeletal muscle function. Mutations of Nav1.4 underlie the pathophysiology of various conditions, including myotonias, paramyotonias and periodic paralyses. The L1436P mutation was been recently recognized as underlying a paramyotonia in some patients. We investigated the effects of the mutation on the current properties.

METHODS HEK283 cells were transfected with plasmids encoding either the wild-type (WT) or mutant channels. The cells were recorded at room temperature using whole-cell patch clamp in a conventional Na⁺-based Ringer. The intracellular solution was Cs gluconate-based.

RESULTS The current through both WT and mutant channels was reversibly abolished by 1 μ M tetrodotoxin and sham transfected cells yielded no inward currents. The mutation had no effect on the activation curve of the current. However, it induced several deficits, including 1) a slowed fast inactivation when pulsing from -100 mV to -10, 0 or +10 mV 2) a faster recovery from fast inactivation (mean tau was 1.9 and 1.2 ms, respectively) 3) a depolarized steady-state inactivation curve, with the mean V_{0.5} shifting from -41 to -33 mV.

CONCLUSION | The L1436P mutation of Nav1.4 induces inactivation deficits which are consistent with those reported previously with other paramyotonia-inducing mutations.

Cardiomyocyte differentiation from iPS cells is delayed following knockout of Bcl-2

Tim Vervliet

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Anti-apoptotic B-cell lymphoma 2 (Bcl-2) regulates a wide array of cellular functions involved in cell death, cell survival decisions and autophagy. Bcl-2 acts by both direct interaction with different components of the pathways involved and by intervening in intracellular Ca²⁺ signalling. The function of Bcl-2 is in turn regulated by post-translational modifications including phosphorylation at different sites by various kinases. Besides functions in cell death and apoptosis, Bcl-2 regulates cell differentiation processes, including of cardiomyocytes, although the signalling pathways involved are not fully elucidated. To further address the role of Bcl-2 during cardiomyocyte differentiation, we investigated the effect of its genetic knockout by CRISPR/Cas9 on the differentiation and functioning of human induced pluripotent stem cells to cardiomyocytes. Our results indicate that differentiation of iPS cells to cardiomyocytes is delayed by Bcl-2 KO, resulting in reduced size of spontaneously beating cells and reduced expression of cardiomyocyte Ca²⁺ toolkit and functionality. These data thus indicate that Bcl-2 an essential protein for cardiomyocyte generation.

POSTER PRESENTATION 1

Adiponectin pathway in a murine model of disuse muscle atrophy: an in vivo study

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INTRODUCTION Skeletal muscle deconditioning results in the development of a Disuse-mediated Muscle Atrophy (DMA) associated to an impaired regeneration potential compromising muscle recovery. Increasing evidence highlights the positive role of the adipo/myokine Adiponectin (Ad) in skeletal muscle. Since Ad pathway was found altered in a murine model of disuse, we hypothesize that such disturbances of Ad biological function could reinforce the loss of muscle mass and the impairment of regeneration potential in DMA.

METHODS A murine model of HindLimb Unloading and Immobilization (HLUI) was designed to minimize the stress that mice may encounter by allowing social interactions and movements with forelimbs thanks to a sliding ring on a rod system. The model was characterized and Ad pathway investigated after 14 days HLUI.

RESULTS In HLUI mice, myofiber CSA was reduced in Soleus and Gastrocnemius muscles. The analysis of myofiber size distribution confirmed a higher number of small sizes fibres in HLUI muscles. The proportion of Low Molecular Weight (MW) Ad forms was decreased in favour of Medium MW multimers in HLUI mice although total Ad plasmatic level was unchanged. AdipoR1 protein level was decreased in HLUI Soleus muscles whereas its expression was unchanged at mRNA and protein level in the Gastrocnemius muscle.

CONCLUSION We optimized a device allowing to mimic DMA in mouse hindlimb muscles. In this model, plasmatic Ad multimer distribution is modified and AdipoR1 protein level is reduced in the slow-twitch muscle soleus. Ongoing studies aim to determine whether Ad may constitute a therapeutic target to counteract DMA.

POSTER PRESENTATION 2

Neuromorphic circuits for undergraduate neurophysiology courses

Shlyonsky V, Dupuis F, de Puelle B, Gall D

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The animal welfare principles are forcing undergraduate teaching to avoid the use of animals. Therefore, many hands-on lab sessions using laboratory animals are progressively replaced by computer simulations. These versatile software simulations permit the observation of the behavior of biological systems under a great variety of experimental conditions. While this versatility is important, computer simulations often work even when a student makes wrong assumptions, a situation that poses its own pedagogical problem. Hands-on learning provides pupils with the opportunity to safely make mistakes and learn organically through trial and error and should therefore still be promoted. We propose an electronic model of an excitable cell composed of different modules representing different parts of a neuron - dendrites, soma, axon and Ranvier node. We describe a series of experiments that allow students to better understand differences between passive and active cell responses and differences between myelinated and demyelinated axons. These circuits also can also be used to demonstrate temporal and spatial summation of signals coming to the neuron via dendrites, as well as the neuron coding by firing frequency. Finally, they permit experimental determination along with theoretical calculations of important biophysical properties of excitable cells, such as rheobase, chronaxie and space constant. This open-source model has been successfully integrated into undergraduate course of the physiology of excitable cells and student feedback assessment reveals that it helped students to understand important notions of the course. Thus, this neuromorphic circuit could be valuable tool for biophysics and neurosciences courses in other universities.