

**BELGIAN SOCIETY OF
PHYSIOLOGY AND PHARMACOLOGY**

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting
Friday, April 28th 2023

PROGRAMME

Venue

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

Local host

**Dr. Roberta Gualdani
UCLouvain
Belgium**

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



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09.15-10.00 Welcome with coffee and tea

Keynote lecture

10.00-11.00 **The Voltage Sensors that Govern CaV1.1 Channel Opening and Skeletal Muscle Contraction**
Riccardo Olcese (University of California Los Angeles, USA)

Oral communications (morning session)

- 11.00-11.15 **A corneal neuralgia TRPV1 mutation increases response to acidic pH and alters agonist sensitization and desensitization**
Roberta Gualdani, Philippe Gailly, Xavier Yerna, Solène Barbeau, Deborah S. Jacobs, Sulayman Dib-Hajj, Stephen G. Waxman (UCLouvain, Massachusetts Eye and Ear, and Yale School of Medicine, USA)
- 11.15-11.30 **The glycolytic enzyme pyruvate kinase M2 (PKM2) functions as a modulator of cytosolic and mitochondrial Ca²⁺ signaling in a cancer cell line**
Fernanda O. Lemos, Ian de Ridder, Shreya Ramesh, Martin D. Bootman, Geert Bultynck, Jan B. Parys (KULeuven, and The Open University, Walton Hall, UK)
- 11.30-11.45 **Unraveling the impact of intracellular BAPTA on cell function and the development of novel intracellular Ca²⁺ chelators**
Femke Speelman-Rooms, Flore Sneyers, Martin Bootman, Geert Bultynck, Steven Verhelst (KULeuven)

11.45-12.00 Selection of peptides for a muscle-targeted delivery of ASO directed against DUX4 mRNAs through complementary approaches in silico, in vitro and in vivo

Maëlle Limpens, Aline Derenne, Carmen Burtea, Sophie Laurent, Alexandre Legrand, Steve Wilton, Alexandra Belayew, Frédérique Coppée, Anne-Emilie Declèves, Alexandra Tassin (UMons)

12.00 – 12.30 Lunch

12.30 – 13.30 Guided Poster Session

Posters:

- **Molecular mechanisms of aneuploidy and glioblastoma aggressiveness induced by Diaph3 loss**
Caren Jabbour, Nicolas Tajeddine, Philippe Gailly, Roberta Gualdani, Olivier Schakman, Farah Issa, Thibaud Parpaite, Xavier Yerna, Solène Barbeau (UCLouvain)
- **A Not-So-Simple Simplification**
Romain Vitello, Hossein Taouba, Donna Pereira Barbon, Nikolay Tumanov, Johan Wouters, Vincent Seutin, Jean-François Liégeois (ULiège and UNamur)
- **Silica nanoparticles inhibit responses to ATP in human airway epithelial 16HBE cells**
Alina Milici, Alicia Sanchez, Karel Talavera (KULeuven)
- **A theoretical framework for polymodal gating of sensory TRP channels**
Inti Zumeta-Dubé, Alina Milici, Enrique Velasco, Karel Talavera (KULeuven)
- **Modulation of the chemosensory cation channel TRPA1 by cholesterol reducing drugs.**
Justyna B. Startek, Alina Milici, Katharina Held, Thomas Voets, Karel Talavera (KULeuven)

Oral communications (afternoon session)

13.30-13.45 Sustained Intermittent Hypoxaemia as a component of COPD pathophysiology: which effect on skeletal muscle?

Lise Paprzycki, Yamina Gourari, Alexandre Legrand, Florence Debacq-Chainiaux, Alexandra Tassin (UMons, UNamur)

13.45-14.00 Serpina3: a novel marker of anthracycline-induced cardiovascular toxicity: from bench to bedside

Dustin Krüger, Matthias Bosman, Hanne Boen, Emeline Van Craenenbroeck, Constantijn Franssen, Pieter-Jan Guns, Charles-Henri Van Assche, Berta Cillero, Leen Delrue, Ward Heggermont, Guido De Meyer (UAntwerp, UZA, M4i Maastricht, OLV Aalst)

14.00-14.15 Conditional deletion of KCC2 impairs synaptic plasticity and both spatial and nonspatial memory

A Kreis, F Issa, X Yerna, C Jabbour, O Schakman, M de Clippele, N Tajeddine, N Pierrot, JN Octave, R Gualdani, P Gailly (UCLouvain)

14.15-14.30 Role of subthreshold membrane potential instabilities in sensory transduction

Enrique Velasco, Julio L. Alvarez, Karel Talavera (KULeuven)

14.30-15:00 Coffee – Tea and Networking

Closing lecture

15.00-15.45 Cytosolic Sodium Keeps a Tight Rein on the Na⁺-Ca²⁺ Exchanger to Tune Cardiac Function

Michela Ottolia (University of California Los Angeles, USA)

ORAL PRESENTATION 1

A corneal neuralgia TRPV1 mutation increases response to acidic pH and alters agonist sensitization and desensitization

Roberta Gualdani¹, Philippe Gailly¹, Xavier Yerna¹, Solène Barbeau¹, Deborah S. Jacobs², Sulayman Dib-Hajj³, Stephen G. Waxman³

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The factors that contribute to pain after nerve injury remain incompletely understood. Laser-assisted in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK), two commonly used surgical techniques to correct myopia, transect the distal axons of trigeminal ganglion neurons within the cornea. After surgery, a subset of patients suffer intense and persistent pain, of unknown origin, described by patients as feeling like shards of glass in their eye.

In order to investigate the contribution of genetic factors in corneal neuralgia, we used whole-exome sequencing analysis of patients reporting persistent pain after refractive surgery, which revealed multiple variants of ion channels including transient receptor potential (TRP) channels. Here we evaluated a TRPV1 variant, p.V527M, found in a 30-year-old woman who had developed corneal pain after LASIK/PRK surgery, reporting an Ocular Surface Disease Index (OSDI) score of 100.

Using patch-clamp and Ca²⁺ imaging we found that V527M shows an enhanced response to acidic pH. In addition, increasing proton concentration induced a stronger leftward shift in the activation curve of V527M compared to WT, resulting in channel activity of the mutant in acidic pH at more physiological membrane potentials. Finally, comparing the responses to consecutive applications of different agonists, we found in V527M a reduced capsaicin-induced desensitization and an increased sensitization by the arachidonic acid metabolite 12-hydroxyheicosatetraenoic acid (12-HETE).

We hypothesize the increased response to protons and enhanced sensitization by 12-HETE in V527M, two inflammatory mediators present in the cornea after tissue damage, may contribute to the pathogenesis of corneal neuralgia after refractive surgery.

ORAL PRESENTATION 2

The glycolytic enzyme pyruvate kinase M2 (PKM2) functions as a modulator of cytosolic and mitochondrial Ca²⁺ signaling in a cancer cell line

Fernanda O. Lemos¹, Ian de Ridder¹, Shreya Ramesh¹, Martin D. Bootman², Geert Bultynck¹ and Jan B. Parys¹

¹Laboratory of Molecular and Cellular Signaling, Dept. Cellular and Molecular Medicine & Leuven Kanker Instituut, KU Leuven, B-3000 Leuven, Belgium ²School of Life, Health and Chemical Sciences, The Open University, Walton Hall, MK7 6AA, UK

INTRODUCTION | Pyruvate kinase M2 (PKM2) is an alternatively spliced variant of pyruvate kinase, highly expressed in cancer cells. PKM2 controls the final, rate-limiting step of glycolysis, contributing to the Warburg effect, and supporting anabolic processes during tumorigenesis. PKM2 also shows non-metabolic roles, such as promoting gene expression, cell cycle progression and cell survival. Recently, our group showed that PKM2 can modulate intracellular Ca²⁺ signaling by interacting with the inositol 1,4,5-trisphosphate receptor (IP3R), thereby decreasing its activity (Lavik et al., 2022, BBA-MCR 1869:119206).

METHODS | We analyzed Ca²⁺ signaling in cytosol and mitochondria of wild-type and PKM2 KO HeLa cells, and compared in those cells ER-mitochondria contact sites as well as mitochondrial characteristics.

RESULTS | Our results demonstrate that PKM2 suppresses IP3R-dependent cytosolic Ca²⁺ signals without interfering with either ER Ca²⁺-store content, store-operated Ca²⁺ entry, or IP3R expression levels. Interestingly, PKM2 also impacts mitochondria at various levels. ER-mitochondrial contact sites are decreased in PKM2 KO cells, mitochondrial membrane potential is increased as is respiration and mitochondrial morphology is altered. Taken together, PKM2 KO resulted in a decreased Ca²⁺ uptake in the mitochondria.

CONCLUSIONS | Our results establish the modulation of mitochondrial properties and mitochondrial Ca²⁺ signaling as a new function of PKM2, which likely contributes to the survival and/or proliferation of cancer cells. Future work will assess the molecular determinants of PKM2 interaction with the IP3R.

This work was supported by grant G0A6919N of the Research Foundation Flanders (FWO) to JBP.

ORAL PRESENTATION 3

Unraveling the impact of intracellular BAPTA on cell function and the development of novel intracellular Ca²⁺ chelators

Speelman-Rooms Femke, Sneyers Flore, Bootman Martin, Bultynck Geert, Verhelst Steven

KULeuven (Laboratory of Molecular and Cellular Signaling/Laboratory of Chemical Biology)

Ca²⁺ signaling has been implicated in virtually all cellular processes, including autophagy, a lysosomal turn-over process in cellular homeostasis. Moreover, Ca²⁺ dysregulation contributes to pathophysiological conditions, such as oncogenesis and neurodegenerative disorders. Much of what is known about Ca²⁺ signaling has been based on the use of pharmacological tools that modulate cellular signals. One such tool that has been extensively used is BAPTA, a fast and high-affinity Ca²⁺ chelator. However, recent studies have shown that BAPTA directly impacts other cellular components and has Ca²⁺-independent actions. Therefore, BAPTA can no longer be considered a reliable means of validating the role of Ca²⁺ in cellular processes and researchers should be cautious in interpreting findings based on the use of BAPTA.

This highlights the need for (i) a re-evaluation of the role of Ca²⁺ in cellular processes such as autophagy, where Ca²⁺ has been implicated largely based on the inhibitory action of BAPTA (ii) the development of improved BAPTA variants. My work aims to meet these existing needs. On the one hand, by comparing BAPTA with low Ca²⁺ -affinity BAPTA variants, I critically examine the exact role of Ca²⁺ signaling in autophagy. On the other hand, I investigate how the molecular structure of BAPTA should be adapted to avoid certain Ca²⁺-independent actions.

Based on these findings, I am developing novel Ca²⁺ chelators that will enable a more accurate identification of Ca²⁺-dependent cellular processes. Ultimately, this work will contribute to a better understanding of intracellular Ca²⁺ signaling in autophagy and potentially other cellular processes.

Selection of peptides for a muscle-targeted delivery of ASO directed against DUX4 mRNAs through complementary approaches in silico, in vitro and in vivo

Maëlle Limpens^{1,2}, Aline Derenne^{1,2}, Carmen Burtea³, Sophie Laurent³, Alexandre Legrand¹, Steve Wilton⁴, Alexandra Belayew¹, Frédérique Coppée², Anne-Emilie Declèves², Alexandra Tassin¹

1 Department of Respiratory Physiology, Pathophysiology and Rehabilitation, 2 Department of Metabolic and Molecular Biochemistry, 3 Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory; Research Institute for Health Sciences and Technology, University of Mons

Facioscapulohumeral dystrophy is an (epi)genetic disease caused by DUX4 aberrant expression in skeletal muscle. DUX4 is expressed during early embryogenesis and in germ lines and is normally repressed in somatic cells. It encodes a transcription factor that alters multiple pathways in skeletal muscle cells leading to FSHD pathophysiology. Currently, there is no treatment available for FSHD. In a therapeutic goal, antisense oligonucleotides (ASOs) directed against DUX4 mRNAs have been developed at UMONS. Our strategy consists in coupling the ASOs with peptides that will ensure the muscle delivery and avoid/or minimize their unspecific clearance after systemic delivery.

Therefore, by screening a phage-display library of linear peptides against either myotubes or a muscle-membrane protein, we selected phage clones that specifically bound to human and mouse muscle surface proteins (MSPep). The peptides encoded by the 4 most promising clones were then synthesized with Rhodamine conjugation for testing in cell cultures in vitro.

Endocytosis of the MSPeps was first investigated in muscle cells compared to hepatocytes and renal cells. In vivo, the efficiency of ASOs targeting DUX4 mRNAs is also tested in the IMEP-DUX4 mouse model in which a DUX4 expression plasmid is electroporated into the tibialis anterior and induces the development of an easily quantifiable muscle lesion. Based on our in vitro and in vivo results, the most efficient MSPeps will be complexed with the most efficient ASOs. Further experiments will aim to evaluate the ability of MSPep-ASOs to target skeletal muscle and deliver efficient ASO against DUX4 mRNA.

Sustained Intermittent Hypoxaemia as a component of COPD pathophysiology: which effect on skeletal muscle?

Lise Paprzycki¹, Yamina Gourari¹, Alexandre Legrand¹, Florence Debacq-Chainiaux², Alexandra Tassin¹

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INTRODUCTION | Chronic Obstructive Pulmonary Disease (COPD) is associated to systemic comorbidities including a skeletal muscle dysfunction. The etiology of COPD muscle dysfunction is multifactorial and involves episodic desaturations leading to a persistent hypoxaemia. Underlying mechanisms need to be clarified but interestingly, an impaired muscle regeneration was suggested.

METHODS | To decipher the specific effect of episodic hypoxaemia on skeletal muscle we used a reductionist mice model exposed to Sustained Intermittent Hypoxaemia (SIH ; FiO₂ : 10%, 8h/day) in a device optimized (i) to avoid movement restriction, (ii) to ensure an homogenous distribution of gaseous flow. Moreover, we limited antioxidant excess by using a specific diet. Muscle structural changes and the expression of myogenic markers are currently investigated in fast and slow-twitch muscles at early and late timepoints.

RESULTS | Our preliminary data on SIH mice showed a decreased expression of the gene encoding Myogenin (Myog) at 7 days with a return to control level at 35 days in the gastrocnemius muscle. At this timepoint, the Cross-Sectional Area (CSA) of the soleus muscle is slightly reduced in SIH mice.

CONCLUSION | We optimized a model allowing to study the specific effect of SIH on skeletal muscle while limiting confounding factors. In this model, our first results suggest an early and transient alteration of myogenic factor expression as well as a slight atrophy taken place over time. Further studies are now needed to assess the contribution of a regeneration defect to hypoxaemia-mediated muscle dysfunction and to investigate underlying molecular mechanisms.

Serpina3: a novel marker of anthracycline-induced cardiovascular toxicity: from bench to bedside

Dustin Krüger, Matthias Bosman, Hanne Boen, Emeline Van Craenenbroeck, Constantijn Franssen, Pieter-Jan Guns, Charles-Henri Van Assche, Berta Cillero, Leen Delrue, Ward Heggermont, Guido De Meyer

University of Antwerp, UZA, M4i Maastricht, OLV Aalst

INTRODUCTION | While survival rates of cancer patients are improving, cardiovascular adverse effects of chemotherapeutics have become a growing concern. For instance, doxorubicin (DOX), a highly effective chemotherapeutic, is known to induce cardiotoxicity in a minority of patients. Until today, it is still challenging to identify patients at risk to initiate cardioprotective therapy early-on. The objective of the present study was to identify functional or molecular markers of cardiotoxicity induced by DOX.

METHODS | Male C57BL6 mice (n=8) were treated with DOX (4mg/kg, weekly i.p. injection) for 6 weeks, and cardiovascular function (left ventricular ejection fraction, LVEF) was longitudinally investigated using ultrasound-imaging. Subsequently, heart tissue was collected after 2&6 weeks for proteomics (liquid-chromatography with mass-spectrometry). In addition, the most promising biomarker identified with proteomics was evaluated in plasma samples of patients treated with DOX with and without cardiotoxicity.

RESULTS | In the mice model DOX induced a consistent reduction of LVEF by 15% after 6 weeks. Proteomic analyses of cardiac samples showed upregulation of a number of proteins, of which serpin3n showed high upregulation in DOX treated animals. Serpin3n, a serine protease inhibitor, has been previously proposed as prognostic marker in cardiovascular diseases. Upregulation of serpin3n was confirmed by RT-qPCR and immunohistochemistry. Similarly, SERPINA3 was increased in plasma of DOX-treated patients (n=14) who developed severe cardiotoxicity (LVEF<50%) compared to controls (n=27).

CONCLUSION | This study is the first to identify increased SERPINA3 levels in DOX-induced cardiotoxicity in both mice and patients. Our data suggested that SERPINA3 could serve as a biomarker.

Conditional deletion of KCC2 impairs synaptic plasticity and both spatial and nonspatial memory

A Kreis, F Issa, X Yerna, C Jabbour, O Schakman, M de Clippele, N Tajeddine, N Pierrot, JN Octave, R Gualdani, P Gailly

Université catholique de Louvain

INTRODUCTION | The postsynaptic inhibition through GABA_A receptors (GABA_AR) relies on two mechanisms, a shunting effect due to an increase of the postsynaptic membrane conductance and, in mature neurons, a hyperpolarization effect due to an entry of chloride into postsynaptic neurons. The second effect requires the action of the K⁺-Cl⁻ cotransporter KCC2 which extrudes Cl⁻ from the cell and maintains cytosolic concentration very low. Neuronal chloride equilibrium seems to be dysregulated in several neurological and psychiatric conditions.

METHODS | We used KCC2 Cre-lox knockdown system to investigate the role of KCC2 in synaptic plasticity and memory formation in adult mice.

RESULTS | Tamoxifen-induced conditional deletion of KCC2 in glutamatergic neurons of the forebrain was performed at 3 months of age and resulted in spatial learning impairment. On brain slices, stimulation of Schaffer collaterals by a theta burst induced a long-term potentiation. The lack of KCC2 did not affect potentiation of field excitatory postsynaptic potentials (fEPSP) measured in the stratum radiatum (dendrites) but increased population spike (PS) amplitudes measured in the CA1 somatic layer, suggesting a reinforcement of the EPSP-PS potentiation. At the cellular level, KCC2 deletion induced a positive shift in the reversal potential of GABA_AR-driven Cl⁻ currents suggesting an intracellular accumulation of chloride subsequent to the downregulation of KCC2. After treatment with bumetanide, an antagonist of the Na⁺-K⁺-Cl⁻ cotransporter NKCC1, spatial memory impairment, chloride accumulation and EPSP-PS potentiation were rescued in mice lacking KCC2.

CONCLUSIONS | This emphasizes the importance of chloride equilibrium and GABA inhibiting ability in synaptic plasticity and memory formation.

Role of subthreshold membrane potential instabilities in sensory transduction

Enrique Velasco, Julio L. Alvarez, Karel Talavera

KU Leuven

INTRODUCTION | Rather than being electrically quiescent while not firing action potentials, peripheral sensory neurons can display marked subthreshold membrane potential instabilities (SMPIs), but the relevance for physiological sensation and molecular bases of this phenomenon remain unknown.

METHODS | We used whole-cell patch-clamp in primary cultured mouse trigeminal (TG) neurons.

RESULTS | Small diameter mouse TG neurons display subthreshold membrane potential transients (SMPTs), whose frequency, amplitude and rates of upstroke and repolarization increase with membrane depolarization induced by artificial current injection and by natural stimuli such as heat, cold, capsaicin and menthol. Morphological and regression analyses show that larger and faster SMPTs actually precede action potentials fired by all stimulation modalities. Ablation of NaV1.9 channels reduce SMPT activity and the firing of action potentials.

CONCLUSION | We hereby demonstrate a causal relationship between SMPTs and AP firing upon exogenous stimulation of sensory neurons and identify, for the first time, molecular determinants of the trigger of SMPTs. These findings unveil SMPTs as crucial regulators of peripheral sensory processing.

POSTER PRESENTATION 1

Molecular mechanisms of aneuploidy and glioblastoma aggressiveness induced by Diaph3 loss

Caren Jabbour, Nicolas Tajeddine, Philippe Gailly, Roberta Gualdani, Olivier Schakman, Farah Issa, Thibaud Parpaite, Xavier Yerna, Solène Barbeau

Université Catholique de Louvain, Institute of Neuroscience

Diaphanous-related formin 3 (Diaph3) belongs to the formin family of dimeric multidomain proteins that are master regulators of actin and microtubule dynamics. It plays a role in the cytoskeleton remodelling, more specifically in cytokinesis and karyokinesis. In the mouse brain, loss of Diaph3 induced aneuploidy followed by massive apoptosis in neural stem cell, leading to microcephaly. Given the potential role of aneuploidy in cancer development, we are interested to unravel whether Diaph3 depletion during gliomagenesis could aggravate glioblastoma (GBM) aggressiveness.

Our results showed that siRNA-mediated depletion of Diaph3 in p53 mutated GBM cells (U251 cells) disrupted microtubules and caused mitotic defects such as multipolar mitosis, lagging chromosomes and micronuclei. Karyotyping showed an increase in aneuploidy in Diaph3-depleted U251 cells. Since it has been shown that abnormalities in chromosomes segregation could trigger DNA damage response, we measured phosphorylation of ATM, Chk2 and the DNA damage marker γ H2AX. We observed that Diaph3 depletion increased the number of γ H2AX foci/cell and phosphorylation of Chk2. In contrast, we did not observe any modification in the phosphorylation of ATM after Diaph3 depletion.

We believe that this study may provide insights into the mechanisms by which Diaph3 prevents aneuploidy in glioblastoma cells but also, more generally, into the role of aneuploidy in gliomagenesis.

POSTER PRESENTATION 2

A Not-So-Simple Simplification

Romain Vitello (1), Hossein Taouba¹, Donna Pereira Barbon (1), Nikolay Tumanov (2), Johan Wouters (2), Vincent Seutin (3), Jean-François Liégeois (1)

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Small-conductance calcium-activated potassium (SK) channels are involved in several physio-pathological pathways. Despite the strong pharmacological interest for their blockade, this therapeutic approach is largely underdeveloped. The classical SK ligand is apamin, a small peptide from bee venom. Non-peptidic small-molecules blockers were also developed, UCL1684 being the strongest but unfit for in vivo assimilation due to size and charges. Other series were designed with basic nitrogens, among them, AG525E1 shows the best results. It is a symmetrical bis-tetrahydroisoquinoline able to cross cellular barriers, but is somewhat weaker than UCL1684. A pharmacophore for the blockade of SK channels was thus defined using apamin and UCL1684 as references. It appeared that 2 positively charged nitrogens separated by 5.6 Å are needed to block SK currents.

To pave the way towards new generations of blockers, we decided to simplify the structure and chiral aspect of AG525E1 while conserving the pharmacophore characteristics. Therefore, we developed a chiral chemical series presenting a tetrahydroisoquinoline moiety with an aliphatic base substituent and a single asymmetric carbon. The enantiopure final products were evaluated in an in vitro binding assay, however, none of the compounds displayed affinity for SK channels.

Consequently, we re-evaluated the binding prerequisites in a new computational study, and we successfully produced a new pharmacophore using a ligand-based approach based on AG525E1 and a series of related structures. For now, this pharmacophore is verified when confronted with other known SK ligands, and also explains the lack of potency of this last series we developed.

POSTER PRESENTATION 3

Silica nanoparticles inhibit responses to ATP in human airway epithelial 16HBE cells

Alina Milici, Alicia Sanchez, Karel Talavera

Laboratory of Ion Channel Research, Dept. of Cellular and Molecular Medicine, KU Leuven

Besides its role in gas exchange, the airway epithelium exerts protective responses against environmental insults. Epithelial cells and sensory neurons in the airway epithelium sense the presence of and the damage induced by various foreign substances and react with protective reflexes, such as enhanced mucociliary clearance, initiation of cough and induction of inflammation. As a result of nanotechnological development, the exposure of the general population to airborne nanomaterials has increased lately, raising concerns about their toxicity. Because of their versatility, silica nanoparticles (SiNPs) are used in multiple manufacturing applications and are among the most produced nanomaterials worldwide. SiNPs affect the airway epithelium function, but the underlying biochemical pathways remain largely unknown. SiNPs have been repeatedly shown to induce release of damage-associated molecular pattern ATP in exposed cells.

We have investigated the effects of SiNPs on the responses of cultured human bronchial epithelial (16HBE) cells to extracellular ATP, using measurements of intracellular Ca^{2+} concentration. Upon ATP stimulation, these cells displayed a concentration-dependent increase in intracellular Ca^{2+} , originating from intracellular stores. SiNPs induced a dose-dependent Ca^{2+} increase and inhibited the Ca^{2+} responses to ATP within minutes of application and at low $\mu\text{g/ml}$ concentrations, which are significantly faster and more potent than those previously reported for the induction of cellular toxicity and pro-inflammatory responses. SiNPs-induced inhibition is independent from the increase in intracellular Ca^{2+} they produce, is largely irreversible and occurs via a non-competitive mechanism.

These findings suggest that SiNPs reduce the ability of epithelial cells to support defensive responses via ATP-dependent signaling.

POSTER PRESENTATION 4

A theoretical framework for polymodal gating of sensory TRP channels

Inti Zumeta-Dubé, Alina Milici, Enrique Velasco, and Karel Talavera

Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven; VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium

INTRODUCTION | The roles of TRP channels as mechanotransducers in physiological conditions remain debated, it has been proposed that most of them can be activated by mechanical stimulation. However, theoretical support for the determinants of mechanosensitivity still lacked.

METHODS | Physical and mathematical formalism for Markov description of gating dynamics in ion channels

RESULTS | The model predicts that the thermal and mechanical sensitivities are determined by the balance between an energy component associated to the protein volume and another associated to the protein-membrane surface. Relatively small structures with thin walls or structures with “long arms” (protuberating) are favored to meet this requirement. The model supports the hypothesis linking the mechanical- and voltage-dependent gating by predicting inverse relationships between the mechanical sensitivity and the gating valence.

CONCLUSION | -The thermal and mechanical sensitivities are determined by the balance between an energy component associated to the channel volume and another associated to the channel/membrane surface. -The model supports the hypothesis linking the mechanical- and voltage-dependent gating by predicting negative relationships between the mechanical sensitivity and the gating valence, in line with previous works -Our model also predicts that amplification of $\gamma_{(p/w)}$ with respect to $\gamma_{(ch/m)}$ is possible (coupling factor dependent), which is an equivalent effect to that obtained by leverages.

POSTER PRESENTATION 5

Modulation of the chemosensory cation channel TRPA1 by cholesterol reducing drugs

Justyna B. Startek, Alina Milici, Katharina Held, Thomas Voets and Karel Talavera

Laboratory of Ion Channel Research (VIB-KU Leuven)

INTRODUCTION | Cholesterol is a bioactive lipid that contributes to the structural stability of signalling hubs in cellular membranes. Cholesterol dysregulation can result in life-threatening cardiovascular diseases, that are commonly treated with cholesterol-inhibiting medications such as statins. However, these drugs can have serious side effects, such as sensory loss, chronic pain and inflammation. TRPA1 is arguably the most versatile sensor of noxious chemicals, thermal and mechanical stimuli and is implicated in a plethora of pain and inflammatory conditions. Further, the effects of statins on sensory TRP channels remain largely understudied.

METHODS | We used intracellular Ca^{2+} measurements and patch-clamp recordings to characterize the effects of statins on TRPA1. We took advantage of species-specific channel variants and used site mutations to inquire about the mechanisms of TRPA1 activation by statins. Fluorescent dyes that are sensitive to alteration of the lipid environment were used to assess the ability of statins to induce mechanical perturbations of the plasma membrane.

RESULTS | We found that all tested statins induce direct, concentration-dependent, and reversible intracellular Ca^{2+} increases, which are abolished by the TRPA1 inhibitor. We propose the mechanism underlying statin-induced activation of mTRPA1 depends on both: direct interactions with a putative menthol binding site and the induction of mechanical perturbations in the plasma membrane.

CONCLUSION | Taken together, the fact that TRPA1 is directly activated by statins, is sensitive to membrane cholesterol levels, and is critically implicated in the pathophysiology of neuropathy, makes it an interesting subject to study the sensory side effects of statins.