BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Friday, October 20th 2017

PROGRAMME

Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
"Espace Roi Baudouin - Atrium"
Rue Ducale / Hertogsstraat 1
1000 Brussels

Local host

Prof. Dr. Wim MARTINET
Department of Pharmaceutical Sciences
University of Antwerp
Universiteitsplein 1
2610 ANTWERPEN-WILRIJK

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Royal Flemish Academy of Belgium for Science and the Arts

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Palace of the Academies
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10.00-10.05 Tribute to a distinguished Ghent University dream team:
Prof. Dr. Johan Van de Voorde and Mr. André Van Baeveghem

Prof. Dr. Vincent SEUTIN – Secretary General of the Belgian Society of Physiology and Pharmacology.

Main Lecture

10.05-11.00 **Autophagy, a guardian against neurodegeneration.**

Prof. Dr. David RUBINSZTEIN – Cambridge Institute for Medical Research, Cambridge – United Kingdom.

Oral Communications

11.00-11.15 I. COORNAERT, G. MARCASSOLI, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen): Macrophage-specific RIP1 deletion reduces necrotic core formation in atherosclerotic plaques of ApoE knockout mice.

11.15-11.30 E. KANIA, B. HARLET, G. ROEST, K. WELKENHUYZEN, E. LOBBESTAEL, V. BAEKELANDT, G. BULTYNCK, J.B. PARYS (KU Leuven)
Role of LRRK2 kinase in Ca^{2+}-dependent autophagy.

11.30-11.45 T. VERVLOESSEM, B. SASI, D. EFREMOV, G. BULTYNCK (KU Leuven, Rome Italy)
BDA-366, a novel Bcl-2 BH4 domain antagonist, induces apoptosis through a Bcl-2 independent mechanism in DLBCL and CLL.

11.45-12.00 L. VANDEN DAELE, C. BOYDENS, J. VAN DE VOORDE (UGent)
Characterization of the retina-mediated relaxation: involvement of pannexins?

12.00-12.15 H. SHAKERI, A. B. GEVAERT, D. M. SCHRIJVERS, G. R.Y. DE MEYER, G. W. DE KEULENAER, K. LEMMENS, V. F. SEGERS (UAntwerpen, UZAntwerpen)
Neuregulin-1 attenuates stress-induced vascular senescence.

12.15-12.30 N. HANNING, J. HEIRBAUT, H. CEULEERS, I. GOOLAERTS, S. FRANCQUE, H. DE SCHEPPER, J. DE MAN, B. DE WINTER (UAntwerpen, UZAntwerpen)
The role of nafamostat mesilate, a serine protease inhibitor, on visceral hypersensitivity in a post-colitis model in the rat.

12.30-14.00 **Lunch – Guided Poster Session – General Assembly**

Posters
(height 120 cm – width 100 cm)

1. W.J. MALAISSE, R. CRUTZEN, R. BEAUWENS (ULBruxelles)
Glucose-induced increase of pancreatic islet cell volume.

2. N. BENARIBA, E. HUPKENS, K. LOUCHAMI, R. DJAZIRI, A. SENER, W.J. MALAISSE (Tlemcen Algeria, ULBruxelles)
Stimulation by *citrullus colocynthis* seed extracts of insulin-producing pancreatic islet cells regeneration in streptozotocin-induced diabetic rats.

3. J. SANCHEZ-ANDRES, R. POMARES, W.J. MALAISSE (Castellan Spain, ULBruxelles)
Effects of carbamoylcholine on electrical activity of mouse pancreatic islet beta cells exposed to a physiological concentration of glucose: short-term associative memory.

4. D. GALL, A. LOBO-ANTUNES, G. DUPONT (ULBruxelles)
Tonic activation of extrasynaptic NMDA receptors decreases neuronal excitability : a modeling study.

5. P. PLAEKE, J.G. DE MAN, A. SMET, H. VAN SPAENDONCK, S. NULLENS, P. JORENS, G. HUBENS, B. DE WINTER (UAntwerpen, UZAntwerpen)
Effect of Intestinal Alkaline Phosphatase on an impaired GI barrier during sepsis: Results in a CLP-induced murine model for sepsis.

6. B. SEITAJ, H. IVANOVA, J.B. PARYS, A. METHNER, G. BULTYNCK (KU Leuven, Mainz Germany)
TMBIM5, a Bax Inhibitor-1-family member: a novel mitochondrial Ca^{2+}-uptake system?

7. M. KERKHOFS, R. LA ROVERE, M. BITTREMIEUX, T. VERVLOESSEM, J.B. PARYS, G. BULTYNCK (KU Leuven)
Triggering cell death through disruption of the Bcl-2/IP3R interaction in cancer cells: discovering the mechanisms via which BIRD-2 kills diffuse large B-cell lymphoma cells.

8. J. IYYATHURAI, N. WANG, C. D'HONDT, J.X. JIANG, L. LEYBAERT, G. BULTYNCK (KU Leuven, UGent, San Antonio USA)
Intramolecular loop/tail interactions involve the SH3 binding domain, controlling Cx43-hemichannel activity.

9. R. M.L. LA ROVERE, M. BITTREMIEUX, H. AKL, K. WELKENHUYZEN, K. DUBRON, M. BAES, A. JANSSENS, P. VANDENBERGHE, K. RIETDORF, G. MORCIANO, P. PINTON, D. EFREMOV, K. MIKOSHIBA, M.D. BOOTMAN, H. DE SMEDT, J. B. PARYS, G. BULTYNCK (KU Leuven, Milton Keynes United Kingdom, Ferrara Italy, Trieste Italy, Saitama Japan)
Constitutive IP_3 signaling underlies the sensitivity of B-cell cancers to the Bcl-2/IP_3 receptor disruptor BIRD-2.

10. A. BUCKINX, D. DE BUNDEL, I. SMOLDERS (VUBrussel)
The functionality of ghrelin-R:D1R interactions in D1R-mediated kindling.

Oral Communications

14.00-14.15 J. VAN DINGENEN, R.A. LEFEBVRE (UGent)
Dimethyl fumarate attenuates murine postoperative ileus independently of heme oxygenase 1.

14.15-14.30 K. JEHASSE, L. MASSOTTE, S. RINGLET, R. VENNEKENS, T. VOETS, D. ENGEL, V. SEUTIN (ULiège, KU Leuven)
Flufenamic acid inhibits the firing of dopamine neurons by a mechanism other than TRP blockade.

14.30-14.45 H. IVANOVA, L.E. WAGNER II, A. TANIMURA, E. VANDERMARLIERE, H. DE SMEDT, L. MARTENS, D. I. YULE, J.B. PARYS, G. BULTYNCK (KU Leuven, UGent, Rochester USA, Hokkaido Japan)
IP_3 and Bcl-2 mutually antagonize their impact on IP_3R function by competing for the ligand-binding domain.

14.45-15.00 F. SNEYERS, T. VERVLOESSEM, M. BITTREMIEUX, G. BULTYNCK (KU Leuven)
The mechanisms underlying the sensitivity of diffuse large B-cell lymphoma cells to intracellular calcium buffering.

15.00-15.15 P. LYBAERT, J. FERREIRA, L. SALKOFF, J. RILEY, C.M. SANTI (St. Louis USA, ULBruxelles)
Membrane potential of human sperm is sensitive to external pH and intracellular Ca^{2+}.

15.15-15.30 G. MONACO, M. MAYBURY, N. NGUYEN, N. HALEMBA, S. VAN DEN HELM, D. MOUJALLED, S. PERCIO, A.M. JABBOUR, P. EKERT, G. DEWSON, A.H. WEI, M. GUTHRIDGE (proposed by G. BULTYNCK) (KU Leuven, Melbourne, Australia)
The metabolic phenotype of acute myeloid leukaemia (AML) arbitrates their response to Venetoclax (ABT-199): opportunities for rational development of combination therapies.

Coffee - Tea

ABSTRACTS

Legend
O = Oral communication
P = Poster

O-01 (11.00-11.15)

Macrophage-specific RIP1 deletion reduces necrotic core formation in atherosclerotic plaques of ApoE knockout mice

I. Coornaert, G. Marcassoli, G.R.Y. De Meyer, W. Martinet

Laboratory of Physiopharmacology, Department of Pharmaceutical Sciences, University of Antwerp, B-2610 Antwerp, Belgium.

Atherosclerosis is a chronic inflammatory disease characterized by the formation of atherosclerotic plaques in medium and large arteries. As the plaque progresses, macrophages undergo necrosis and release their cytoplasmic content in the centre of the plaque, thereby forming a necrotic core. Further expansion of the necrotic core promotes plaque instability and rupture. Hence, targeting macrophage death is a promising strategy to stabilize atherosclerotic plaques. Recently, necroptosis was discovered as a form of regulated necrosis. The formation of a RIP1 and RIP3 pro-necrotic complex, called the necrosome, is a crucial step in the induction of necroptosis. This study aimed to investigate the impact of a macrophage specific RIP1 deletion on atherogenesis by crossbreeding $RIP1^{F/F}$ $LysMCre^{+}$ and $RIP1^{+/+}$ $LysMCre^{+}$ mice with $ApoE^{-/-}$ mice. $RIP1^{F/F}$ $LysMCre^{+}$ $ApoE^{-/-}$ (n=20) and $RIP1^{+/+}$ $LysMCre^{+}ApoE^{-/-}$ (n=20) mice were fed a western-type diet for 16 weeks to induce plaque formation. Although plasma cholesterol level, body weight and heart weight were not significantly altered in $RIP1^{F/F}$ $LysMCre^{+}$ $ApoE^{-/-}$ mice as compared to $RIP1^{+/+}$ $LysMCre^{+}$ $ApoE^{-/-}$ mice, the plaque area in $RIP1^{F/F}$ $LysMCre^{+}$ $ApoE^{-/-}$ mice was significantly decreased (72±7 vs. 150±12 $x10^{3}$ μm^{2}, $p<0.0001$). This effect was associated with a reduction of necrotic core size (1.5±0.8 vs. 6.3±1.0 %, p=0.0005). The collagen deposition in the plaques was not affected (26±4 vs 24±3 %, p=0.687). Similarly, the thickness of the fibrous cap in function of the plaque thickness was not altered (0.03±0.02 vs. 0.05±0.01, p=0.245). However, a macrophage-specific RIP1 deletion resulted in a significantly decreased macrophage content (2.1±0.3 vs. 4.3±0.8 %, p=0.0099). Taken together, these data demonstrate that macrophage RIP1 contributes to atherogenesis.

O-02 (11.15-11.30)

Role of LRRK2 kinase in Ca^{2+}-dependent autophagy

E. Kania[1], B.Harlet[1], G. Roest[1], K. Welkenhuyzen[1], E. Lobbestael[2], V. Baekelandt[2], G. Bultynck[1], J.B. Parys[1]

[1]Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, K.U.Leuven, O&NI Herestraat 49 bus 802, 3000 Leuven, Belgium.
[2]Research Group for Neurobiology and Gene Therapy, Department of Neurosciences, Kapucijnenvoer 33 blok i - box 7001, 3000 Leuven, Belgium.

Mutations in Park8, encoding for LRRK2 (Leucine-Rich Repeat Kinase 2), are the most common genetic determinants in Parkinson's Disease, but LRRK2 is also linked to cancer and Crohn's disease. LRRK2 plays a physiological role in multiple cellular processes, including cytoskeleton remodeling, proliferation, vesicular trafficking and autophagy. Autophagy modulation by LRKK2 kinase seems to be mediated by Ca^{2+} signaling, involving Ca^{2+} release from acidic organelles and endoplasmic reticulum (ER), followed by activation of the CaMKKβ-AMPK pathway. Here we investigate the impact of LRRK2 kinase and of LRRK2 kinase inhibitors on Ca^{2+} handling, on basal autophagy and on starvation-induced autophagy, which we previously demonstrated to be Ca^{2+}-dependent. In our study we show that in neuroblastoma SH-SY5Y cells overexpressing LRRK2, this kinase is phosphorylated on Ser935 and autophosphorylated on Thr1410 during nutrient and amino acid starvation. A similar effect was observed in wild type mouse embryonic fibroblasts (wt MEFs), expressing a high endogenous LRRK2 level. Interestingly, Ser935 was significantly more phosphorylated in MEFs in which both catalytic subunits of AMPK were knocked out (DKO MEFs), largely defective in starvation-induced autophagy. To investigate whether LRRK2 is activated in a Ca^{2+}-dependent manner, we administrated BAPTA-AM which together with autophagy inhibition, decreased basal and starvation-induced phosphorylation of LRRK2 Ser935 in both wt and DKO MEFs. On the other hand, starvation-induced autophagy in wt MEFs was not antagonized by the LRRK2 kinase inhibitors GSK2578215A and LRRK2-IN-1, known to induce LRRK2 Ser935 dephosphorylation. These both inhibitors however stimulated basal mTOR- independent autophagic flux as measured by LC3 lipidation. Additionally, upon LRRK2 kinase inhibition with GSK2578215A we observed a remodeling of ER, displaying a higher Ca^{2+} content than untreated cells. Finally, an increased level of autophagy was also observed in SH-SY5Y cells expressing LRRK2 bearing mutations in four serine residues clustered together (S910A, S935A, S955A, S973A). Taken together our data suggest a complex role of LRRK2 kinase in mTOR-dependent (starvation) and mTOR independent (LRRK2 kinase inhibitors) autophagy. Ca^{2+} seems to play a role upstream to LRRK2, contributing to its activation, but Ca^{2+} is also likely to mediate downstream effects of LRRK2 kinase, as observed by the ER Ca^{2+} store remodeling occurring after LRRK2 inhibition.

O-03 (11.30-11.45)

BDA-366, a novel Bcl-2 BH4 domain antagonist, induces apoptosis through a Bcl-2 independent mechanism in DLBCL and CLL

Tamara Vervloessem[1], B. Sasi[2], D. Efremov[2], G. Bultynck [1]

[1] KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut (LKI), Leuven, Belgium.[2] Molecular Hematology, International Center for Genetic Engineering & Biotechnology, Rome, Italy

Anti-apoptotic Bcl-2 is an essential inhibitor of apoptosis. Via its hydrophobic cleft, Bcl-2 is able to inhibit the activation of pro-apoptotic family members thereby inhibiting Bax-dependent apoptosis. On the other side, the Bcl-2 homology (BH) 4 domain of Bcl-2 operates at the level of the inositol 1,4,5 trisphosphate receptor (IP_3R) thereby inhibiting aberrant Ca^{2+} fluxes. Hence, to ensure their survival and support their proliferative signaling, many cancer cells upregulated anti-apoptotic Bcl-2. Therefore, Bcl-2 has become a prime target for anti-cancer therapy. Recently a small molecule BDA-366 has been developed to target the BH4 domain of Bcl-2 (Han, Park et al. 2015), thereby converting Bcl-2 into a pro-apoptotic protein and induces Bax-dependent apoptosis in lung cancer and multiple myeloma. The toxicity towards BDA-366 in these cancer cells correlated with the Bcl-2-expression level. We investigate the effect of BDA-366 in diffuse large B-cell lymphoma (DLBCL) cells. DLBCL cells showed a very heterogeneous response towards BDA-366 and this independent of Bcl-2. These results were also confirmed in primary chronic lymphocytic leukemia (CLL). Moreover, BDA-366 induced Bax-mediated, but Ca^{2+} independent apoptosis in DLBCL cell lines. Additionally, in DLBCL and CLL, we noticed that BDA-366 reduced Mcl-1 protein levels, thereby significantly shortened the half-life of Mcl-1, without affecting the stability of other anti-apoptotic proteins like Bcl-2 and Bcl-XL. In conclusion, BDA-366 induced apoptosis in a collection of DLBCL and primary CLL cell lines . Moreover, the primary mechanism of BDA-366 induced cell death seems to not correlate with the Bcl-2-expression levels in DLBCL nor in CLL cells, challenging the originally proposed mechanism of action using SCLC cells. Instead, BDA-366 decreased the Mcl-1 levels, potentially enhancing the sensitivity towards venetoclax. Hence, these findings provide a strong rationale to implement BDA-366 as a novel anti-cancer therapy to overcome therapeutic resistance.

O-04 (11.45-12.00)

Characterization of the retina-mediated relaxation: involvement of pannexins?

L. Vanden Daele, C. Boydens, J. Van de Voorde

Department of Pharmacology – Vascular Research Unit, Ghent University, Ghent, 9000, Belgium

The retinal relaxing factor (RRF) is a continuously released factor from the retina that causes vasorelaxation and which identity and potential role in physiology remain largely unknown. In this study experiments were performed to find out whether the RRF is preferentially active on retinal arteries and whether the RRF is released from avascular retinas. Also the influence of serotonin, glutamate and L-cysteine, and of inhibition of the cytochrome P450 and cyclooxygenase pathway on the RRF-induced relaxation was investigated. Isometric tension measurements were performed on isolated mice femoral or bovine retinal arteries to study the vasorelaxing effect of the RRF by bringing them in close contact with bovine, mice or chicken retinas or by dripping a RRF-containing solution on them. RRF-induced relaxations were comparable, independent of the type of retina or artery studied, and avascular chicken retinas were able to induce vasorelaxation. The presence of serotonin, glutamate or L-cysteine did not alter the RRF response and neither did the inhibition of the cytochrome P450 pathway or the cyclooxygenase pathway. Surprisingly, flufenamic acid was found to substantially influence the retina-induced relaxation. The observed enlargement is probably due to its inhibitory effect on pannexin 1 as it is also found with carbenoxolone and probenecid. So it is concluded that the RRF has no selectivity for retinal arteries and that also avascular retinas release the RRF. Serotonin, glutamate and L-cysteine, and the inhibition of the cytochrome P450 and cyclooxygenase pathway have no influence on the RRF-induced relaxation. The enlarged RRF-induced relaxation in the presence of flufenamic acid, carbenoxolone and probenecid suggests a potential role of pannexin 1 in the retina-induced vasorelaxation.

O-05 (12.00-12.15)

The protective role of Neuregulin-1 and its receptor ErbB4 in hyperglycemia-induced vascular senescence

H. Shakeri[1], A.B. Gevaert[1,2,3], D.M. Schrijvers[1], G.R.Y. De Meyer[1], G.W. De Keulenaer[1], K. Lemmens[1,4], V.F. Segers[1,2,4]

[1]Laboratory of Physiopharmacology, University of Antwerp, Universiteitsplein 1, 2610, Antwerp, Belgium, [2]Department of Cardiology, Antwerp University Hospital (UZA), Belgium, [3]Laboratory for Cellular and Molecular Cardiology, Department of Cardiology, University Hospital Antwerp, Belgium, [4]Contributed equally

Cardiovascular ageing is a key process that determines life expectancy and quality of life of the elderly. Cellular senescence, a state of irreversible cell cycle arrest, is described as an important contributor to aging due to the accumulation of damaged cells. Targeting cellular senescence can potentially prevent age-related cardiovascular diseases. In this study, we investigated the effect of neureguline-1 (NRG-1) on hyperglycemia-induced vascular senescence. NRG-1 is an epidermal growth factor with powerful cardioprotective and anti-atherosclerotic effects but its role in cellular senescence remains unexplored. To study the effects of recombinant human NRG-1 (rhNRG-1) on hyperglycemia-induced vascular senescence in vivo, C57BL/6 mice were rendered type 1 diabetic with streptozotocin (STZ, 60 µg/kg, 5days) and randomized to receive rhNRG-1 (20 µg/kg) or vehicle. In all diabetic mice, a significant induction of cell senescence in aortic endothelial and vascular smooth muscle cells (SMCs) was observed using the senescence-associated-β-galactosidase (SA-β-gal) staining and Western blot analysis for cell cycle regulator proteins, acetyl-p53 and p21. rhNRG-1 treatment significantly attenuated hyperglycaemia-induced senescence in the aorta. Next, aortic SMCs isolated from SMC-specific ErbB4 deficient mice (ErbB4 $^{F/+}$ SM22α cre+) showed earlier cellular senescence *in vitro* compared to wild type (ErbB4$^{+/+}$ SM22α cre+) SMCs, using SA-β-gal staining and Vindelov method for cell cycle phase. ErbB4$^{F/+}$ SM22α cre+ mouse and its genetically normal littermate (ErbB4$^{+/+}$ SM22α cre+) were rendered diabetic with STZ. Diabetic ErbB4$^{F/+}$ SM22α cre+ mice showed significantly more vascular senescence than diabetic wild type littermates. Also, diabetic ErbB4$^{F/+}$ SM22α cre+ mice have a shorter life expectancy compared to diabetic ErbB4$^{+/+}$ SM22α cre+ mice.This study is the first to explore the role of the cardioprotective growth factor NRG-1 in vascular senescence. Our data demonstrate that NRG-1 markedly inhibits senescence in the aorta of diabetic mice *in vivo*. Consistently, deficiency in the NRG-1 receptor ErbB4 in vascular smooth muscle cells provokes cellular senescence *in vitro* as well as *in vivo*.

O-06 (12.15-12.30)

The role of nafamostat mesilate, a serine protease inhibitor, on visceral hypersensitivity in a post-colitis model in the rat

N. Hanning, J. Heirbaut, H. Ceuleers, I. Goolaerts, S. Francque, H. De Schepper, J. De Man, B. De Winter

University of Antwerp, University Hospital of Antwerp

Irritable bowel syndrome (IBS) is a functional disease, characterized by changes in defecation and abdominal pain. Serine proteases are hypothesized to contribute to visceral hypersensitivity in IBS, but their exact role is still unknown. The aim of this study was to investigate the effect of the serine protease inhibitor nafamostat mesylate (NM) on visceral hypersensitivity in a post-inflammatory rat model for IBS. Colitis was induced in male Sprague-Dawley rats with a trinitrobenzene sulphonic-acid (TNBS) enema, while controls received 0,9% NaCl. Colonoscopy was performed on day 3 to confirm the presence of colitis, and repeated every 4 days from day 10 until endoscopic healing of the mucosa was observed. Visceral sensitivity was assessed by quantifying the visceromotor responses (VMRs) to colorectal distension. NM (0,1-1-10 mg/kg) or its vehicle was injected intraperitoneally 30 min prior to VMR measurements. Inflammatory parameters (colonoscopy, macroscopy, microscopy and myeloperoxidase activity) were used to confirm the post-inflammatory status of the colon. VMR data were analysed by the generalized estimating equation model, followed by LSD post-hoc test when appropriate. Inflammatory parameters were analysed by two-way ANOVA, followed by SNK post-hoc test when appropriate. All TNBS-rats developed colitis by day 3. Their post-inflammatory status was confirmed at the day of the VMR (day 13-21). Visceral hypersensitivity was present in vehicle-treated post-colitis rats, demonstrated by significantly higher VMRs compared to controls. NM significantly reduced visceral hypersensitivity, showing the best results at the lowest dose used (0.1 mg/kg) with an inverse doserelation profile. NM did not modify visceral sensitivity in controls. Our findings indicate that the serine protease inhibitor NM decreases post-inflammatory hypersensitivity in a rat model for IBS. Therefore, we suggest that serine proteases encompass a new potential target in the treatment of visceral pain in IBS patients.

O-07 (14.00-15.15)

Dimethyl fumarate attenuates murine postoperative ileus independently of heme oxygenase 1

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Heymans Institute of Pharmacology, Ghent University, Ghent, 9000, Belgium

Induction of heme oxygenase 1 (HO1), which degrades heme to ferrous iron, CO and biliverdin, is partially responsible for the anti-inflammatory effect of CO-releasing compounds in murine postoperative ileus (POI). Dimethyl fumarate (DMF) is believed to exert its immunosuppressive and neuroprotective effects via HO1 induction and is already used in clinical practice to treat multiple sclerosis. The effect of DMF on the intestinal inflammation and on the delay in gastrointestinal transit caused by POI was therefore studied. POI was induced in C57Bl6J mice by compressing the small intestine (intestinal manipulation; IM) for 5 min and DMF was administered intragastrically (i.g.; 100 mg/kg) or intraperitoneally (i.p.; 30 mg/kg) 24 h before IM. Intestinal transit was assessed using fluorescent imaging and inflammatory parameters were measured in the intestinal muscularis. Pre-treatment with DMF via both i.g. and i.p. administration improved the delayed transit seen after IM and significantly reduced the increased IL-6 levels in the muscularis; the leukocyte infiltration (myeloperoxidase activity) was only significantly reduced with i.p. DMF. At 12 and 24 h after administering DMF in non-operated animals, no increase in intestinal HO1 protein level was measured. IM per se caused a significant increase in intestinal HO1 level but this effect was not magnified with DMF. These results indicate that both i.g. and i.p. administration of DMF prevent delayed intestinal transit and reduce inflammation upon IM, independently of intestinal HO1 induction. Possible inhibition by DMF of the proinflammatory NF-κB pathway is actually analyzed via western blot and immunohistochemistry.

O-08 (14.15-14.30)

Flufenamic acid inhibits the firing of dopamine neurons by a mechanism other than TRP blockade

K. Jehasse[1], L. Massotte[1], S. Ringlet[1], R. Vennekens[2], T. Voets[2], D. Engel[1], V. Seutin[1]

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Midbrain dopamine (DA) neurons are slow intrinsic pacemaker cells. This slow (1-3 Hz) pacemaking activity results from a spontaneous depolarization between spikes, involving a small inward flux of Na^+ and/or Ca^{2+}. Because of technical reasons, the origin of this inward current is still unknown. One potential candidate is the family of transient receptor potential (TRP) channels. It has been suggested that a TRPM subtype (M2 or M4) is involved in the firing of DA neurons because the TRP blocker flufenamic acid (FFA) inhibits the pacemaking activity. We tried to reproduce this observation using extracellular and whole-cell recordings in juvenile and adult rat brain slices. In extracellular experiments, as expected, 100 µM–FFA completely inhibited the firing of DA neurons (n = 9). This effect was partially reversible. Consistent results were obtained in whole-cell experiments in the current clamp mode. In addition, we observed a significant hyperpolarization of DA neurons to about -60 mV. In voltage clamp experiments, FFA produced an outward current at -50 mV (Control: $I_{holding}$ = -41,3 ± 9,8 pA, n = 7; FFA: $I_{holding}$ = 10,6 ± 18,9 pA, n = 7; p = 0,005) and markedly increased the cell conductance as measured by a step from -50 to -60 mV ($G_{control}$ = 1,55 ± 0,1 nS, n = 7; G_{FFA} = 4,28 ± 0,85 nS, n = 7; p = 0,02). These results are not consistent with closure of ion channels, such as TRP blockade, but rather suggest an opening of K^+ or Cl^- channels by the drug. Further experiments are ongoing to discriminate between these two possibilities.

O-09 (14.30-14.45)

IP_3 and Bcl-2 mutually antagonize their impact on IP_3R function by competing for the ligand-binding domain

H. Ivanova[1], L.E. Wagner II[2], A. Tanimura[3], E. Vandermarliere[4] , H. De Smedt[1], L. Martens[4], D.I. Yule[2], J.B. Parys[1], G. Bultynck[1*]

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Anti-apoptotic Bcl-2 proteins have emerged as critical regulators of intracellular Ca^{2+} dynamics by directly targeting and inhibiting the IP_3 receptors (IP_3Rs), intracellular Ca^{2+}-release channels. Yet, the mechanism by which Bcl-2 proteins inhibit IP_3R function and whether IP_3R inhibition by Bcl-2 can be modulated by the strength of agonist/IP_3 signaling remained unknown. Here, we show that inhibition of IP_3R activity by Bcl-2 was alleviated by high concentrations of IP_3 or agonist in permeabilized cells and in single-channel recordings or in intact cells, respectively. This could be attributed to the binding of Bcl-2's BH4 domain to the ligand-binding domain (LBD) of the IP_3R, shown experimentally and fitting *in silico* models. *In silico* analysis revealed steric hindrance by IP_3 of the Bcl-2 binding to LBD. Consistent with this, the binding of Bcl-2 and its BH4 domain to the LBD was antagonized by IP_3 or adenophostin A. *Vice versa*, Bcl-2 or its BH4 domain interfered with IP_3 binding to the IP_3R or to the LBD, respectively. Thus, by competing for the LBD, IP_3 and Bcl-2 mutually affect their impact on IP_3R function: high [IP_3] prevents IP_3R inhibition by Bcl-2 and Bcl-2 prevents IP_3 binding to the IP_3R. This study provides a novel mechanism for IP_3R inhibition by Bcl-2 and reveals that the IP_3R-inhibitory properties of Bcl-2 are counteracted by high concentration of IP_3 or strong agonist stimulation. *Vice Versa*, Bcl-2 negatively regulates IP_3R activity by competing with IP_3 for the ligand-binding domain, reducing channel activity.

O-10 (14.45-15.00)

The mechanisms underlying the sensitivity of diffuse large B-cell lymphoma cells to intracellular calcium buffering

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One of the hallmarks of lymphoid malignancies like diffuse large B-cell lymphoma (DLBCL) is anti-apoptotic Bcl-2 overexpression, which allows cancer cells to escape from oncogenic stress by neutralizing pro-apoptotic Bax/Bak and Bim. At the endoplasmic reticulum (ER), Bcl-2 inhibits IP3Rs, suppressing pro-apoptotic Ca^{2+} release. ABT-199 (venetoclax), a BH3-mimetic, selectively binds and inhibits Bcl-2. Previous research revealed that ABT-199 did neither affect intracellular Ca^{2+} dynamics nor alleviated IP_3R inhibition by Bcl-2. Yet, apoptosis was significantly increased by combining venetoclax with the intracellular Ca^{2+} chelator BAPTA-AM. Here, we aimed to elucidate the mechanisms underlying this synergism by examining the link between intracellular Ca^{2+} buffering, Bcl-2-family members and apoptosis in different DLBCL cell lines. BAPTA-AM treatment induced apoptotic cell death in OCI-LY-1 and SU-DHL-4, although OCI-LY-1 cells appeared more sensitive to BAPTA-AM than SU-DHL-4 cells. The effect of BAPTA-AM on the expression of different pro- and anti-apoptotic Bcl-2-family members was assessed. BAPTA-AM resulted in a complete and rapid loss of Mcl-1, preceding the cell death occurrence in these cells. These results were also confirmed in H929 cells, a Mcl-1-dependent cell line. It is well established that Mcl-1 expression is driven by the mammalian target of rapamycin (mTOR) signaling. We validated this using Torin1, an mTOR inhibitor, which indeed led to a decrease in Mcl-1. Excitingly, BAPTA-AM treatment resulted in a rapid loss of the phosphorylation of p70S6 kinase phosphorylation, a substrate of mTOR. Nevertheless, further work is needed to decipher B-cell cancer cells' addiction to basal Ca^{2+} signaling for their survival and to understand how buffering of basal Ca^{2+} by itself results in cell death and sensitizes cancer cells to BH3-mimetic drugs through Mcl-1 downregulation.

O-11 (15.00-15.15)

Membrane potential of human sperm is sensitive to external pH and intracellular Ca^{2+}

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In the female genital environment, sperm undergo a process essential for fertilization called capacitation, characterized by a change in the intracellular pH, membrane hyperpolarization and a rise in the intracellular calcium concentration. In order to understand the regulation of membrane potential in human sperm, we measured the membrane potential using $DiSC_3$-(5) in human sperm cell populations under different experimental conditions. The average membrane potential of human sperm is -48.31 mV ± 6.56 at pH 7.4 in non-capacitating conditions. This value more positive than the potassium equilibrium potential (E_K), suggests that the sperm resting membrane potential must be determined by other ionic permeabilities in addition to a potassium permeability. Voltage measurements were conducted at different external K^+ concentrations and the data were fitted to the Goldman-Hodgkin-Katz equation. This analysis revealed a significant membrane permeability to Cl^- and Na^+, relative to the K^+ permeability. Incubation of human sperm in a more alkaline environment induced a membrane hyperpolarization. Likewise, the addition of the calcium ionophores A23187 8 µM or Ionomycin 10µM was associated with a hyperpolarization. We incubated the samples with 2 K^+ channels inhibitors, 100 µM Quinidine or 5 mM Tetra-Ethyl-Ammonium (TEA). Although no effect was detected with the addition of TEA, a marked depolarization was observed in the presence of Quinidine. These data suggest that, similar to the mouse model, SLO3 may play an important role in the control of human sperm membrane potential. This study also demonstrated a large variation in how sperm samples from different individuals responded to increasing pH. Additionally, this study lays the groundwork for a detailed study on whether individual genetic variation is a factor in human sperm's ability to undergo membrane hyperpolarization, which might alter the fertilization potential of human sperm.

O-12 (15.15-15.30)

The metabolic phenotype of acute myeloid leukemia (AML) arbitrates their response to Venetoclax (ABT-199): opportunities for rational development of combination therapies.

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Acute Myeloid Leukemia (AML) is an aggressive, genetically heterogeneous adult leukaemia that, to date, lacks effective therapies. Venetoclax/ABT-199, a recently developed inhibitor of the pro-survival BCL-2 protein, has shown antineoplastic activity in some cancers including AML. However, the response rate to Venetoclax in Phase I/II AML trials is still low (approximately 20%). Therefore, it will be important to characterize the molecular basis for AML resistance to Venetoclax as well as rationally develop more effective combination therapies. Cellular energy production is largely governed by two key metabolic pathways: Glycolysis and mitochondrial oxidative phosphorylation (OXPHOS). The aberrant reprogramming of cellular bioenergetics enables tumor cells like AML to obtain the macromolecular precursors and energy needed for rapid growth. We hypothesized that the inherent resistance of AML cells to Venetoclax/ABT-199 is due to their unbalanced reliance on either OXPHOS or Glycolysis and that their altered metabolic dependencies could be selectively targeted. Our data show that the selective targeting of Bcl-2 using Venetoclax/ABT-199 results in a rapid and BAX-dependent downregulation of OXPHOS that is independent of or complementary to its ability to induce apoptosis. On the other hand, inhibitors of oncogenic tyrosine kinases (e.g. FLT3-ITD) or of their downstream target, phosphatidyl inositol 3-kinase (PI3K), impair glycolysis in AML cells. We have identified a direct relationship between the metabolic profile of AML cells and their susceptibility to the induction of cell death by Venetoclax/ABT-199 and PI3K inhibitors. Thus, the metabolic profile of an AML sample may be informative in terms of their drug sensitivities. Finally, the combined inhibition of glycolysis and OXPHOS is highly synergistic at inducing apoptosis in AML cells in vitro, and has anti-leukemic activity in our preclinical mouse xenograft model in the absence of detectable toxicity. Overall, our work suggests previously unknown roles for the Bcl-2 family members in cell metabolism. Furthermore, our findings highlight the potential for targeting metabolic vulnerabilities in AML by rationally combining clinically approved agents that are able to impair oxidative phosphorylation with those that target glycolysis.

P1

Glucose-induced increase of pancreatic islet cell volume

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Tannic acid and the more specific inhibitor of anoctamin 1 T-AO1 inhibit glucose-induced insulin release from rat pancreatic islets. The present study aimed at investigating whether glucose and these agents also affect the volume of dispersed rat islet cells. A rise in glucose concentration from 2.8 to 16.7 mM first caused up to the 20th min of incubation a rapid and progressive increase in cell volume, no further increase in cell volume being observed thereafter. After 15 min preincubation at the low glucose concentration and at the 10th min of incubation, a modest or pronounced increase in cell volume was already noticed in cells exposed during both preincubation and incubation to T-AO1 or tannic acid, respectively. From the 10th to 90th min of incubation, a further progressive increase in cell volume was observed in the cells exposed to an anoctamin 1 inhibitor, the slope of the regression line between cell volume and the logarithmic values of incubation time being significantly higher than in the absence of such an inhibitor. The elevation of these lines was also significantly higher in the presence of tannic acid as compared to T-AO1. These findings are compatible with the view that the increase in cell volume provoked by a rise in glucose concentration and likely attributable to the intracellular accumulation of glucose metabolites is, under physiological conditions, opposed by the gating of anoctamin 1 channels possibly caused by the glucose-induced increase in cytosolic Ca^{2+} concentration.

P2

Stimulation by *citrullus colocynthis* seed extracts of insulin-producing pancreatic islet cells regeneration in streptozotocin-induced diabetic rats

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Streptozotocin-induced diabetic rats received one week after administration of streptozotocin and for the ensuing 3 weeks daily intraperitoneal injection of either saline, glibenclamide or five different extracts prepared from *Citrullus colocynthis* seeds. The percent area occupied by islets in sections of the pancreatic gland stained for insulin averaged 3.5 ± 0.6 in the STZ rats injected either with saline or a defatted aqueous extract, 10.7 ± 1.1 in the rats injected with glibenclamide or either an ethyl acetate or n-butanol extract and 44.9 ± 12.0 in rats injected with either an untreated aqueous or H_2O-methanol extract, these three mean values being significantly different from one another. In the seven groups of rats under consideration, there was a highly significant negative correlation between the logarithmic values for the mean percent islet area and corresponding glycemia measured after overnight starvation at the end of the experiments. It is proposed that the regeneration of insulin-producing cells provoked by selected *Citrullus colocynthis* extracts is attributable, to a large extent, to an improvement of glucose homeostasis and justifies further investigation on the possible use of these extracts in a therapeutic perspective.

P3

Effects of carbamoylcholine on electrical activity of mouse pancreatic islet beta cells exposed to a physiological concentration of glucose: short-term associative memory

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The present study documents that carbamoylcholine stimulates electrical activity of mouse pancreatic islet β-cells exposed to a physiological concentration of glucose. It also reveals that such a stimulation is associated with a short-term memory phenomenon, as documented by an increased bioelectrical response to a rise in extracellular glucose concentration from 8.0 to 12.0 mM lasting for 1 minute after exposure of the cells to the cholinergic agent. The latter phenomenon was apparently mediated by muscarinic receptors, being suppressed by atropine. It failed to occur when no glucose was present in the perfusate at the time of carbamoylcholine administration. However, at that time, the substitution of the hexose by glyceraldehyde, 2-ketoisocaproate and, to a lesser extent, L-leucine or palmitate allowed the occurrence of the memory phenomenon. This temporal potentiation of sensitivity was suppressed when diazoxide was present in the perifusate at the time of carbamoylcholine administration, whilst being again operative when the cholinergic agent was administered in the presence of tolbutamide, indicating the relevance of the (in)activation of ATP-sensitive K^+ channels to the occurrence of the memory phenomenon. The latter phenomenon apparently represented a Ca^{2+}-sensitive process. It did not seem to involve *de novo* protein biosynthesis, inhibition of cAMP phosphor-diesterase or activation of protein kinase C.

P4

Tonic activation of extrasynaptic NMDA receptors decreases neuronal excitability : a modeling study

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Amyloids β (Aβ) are a hallmark of Alzheimer's disease. They affect the communication between neurons. They can also bind to neuronal targets and thereby affect both intracellular signalling and neuronal electrical activity. During the onset of Alzheimer's disease, a positive feedback loop between Aβ40/42 and cytosolic calcium is thought to accelerate the progression of the disease. If intracellular calcium and Aβ reinforce themselves through this mechanism, one would expect that the neurons targets of Aβ may display an altered electrical activity caused by the increase in cytoplasmic calcium as it is known that there is a tight coupling between calcium dynamics and the electrical excitabilty. The aim of this work is to test this assumption when considering one of the target of Aβ, the activity of extrasynaptic NMDA receptors. Our theoretical model is a simple description of neuronal electrical activity based on the Hodgkin-Huxley like formalism, including a term that corresponds to the activity of the NMDA receptor and a cytosolic calcium compartment. When the tonic activity of extrasynaptic NMDA receptors is increased, neurons are less excitable. This is a counterintuive result as NMDA receptors exert an excitatory effect. Further analysis show that this inhibitory effect is due to the activation of calcium-dependent potassium channels, which hyperpolarize the neurons. Activation of extrasynaptic NMDA receptors also provokes a marked increase in intracellular calcium concentration, thus reinforcing the feed-forward relation between Aβ production and calcium.

P5

Effect of Intestinal Alkaline Phosphatase on an impaired GI barrier during sepsis: Results in a CLP-induced murine model for sepsis

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Sepsis is a frequent and severe medical condition that can be caused by any infection or proinflammatory state and results in a systemic inflammatory response. The gastrointestinal tract is highly involved in the pathogenesis of sepsis, as intestinal inflammation and disturbed intestinal permeability can be the initiator or maintaining factor for sepsis. While sepsis is responsible for approximately six to nine million deaths worldwide every year, other than antibiotics and supportive measures, no specific therapy is currently available. Intestinal alkaline phosphatase (IAP) is an alkaline phosphatase subtype ubiquitously expressed in the proximal part of the gastrointestinal tract. It has been associated with the regulation of the lipid absorption and homeostasis of the bicarbonate secretion. Recently, due to its ability to detoxify LPS and UDP, this molecule became of interest in the domain of inflammatory bowel disorders and sepsis. Additionally, IAP has been presumed to promote a healthy intestinal microbiome. Based on these effects, we hypothesized IAP could potentially reduce both intestinal permeability and inflammation during sepsis. In male OF-1 mice (n=12/group) we performed a caecal ligation and puncture technique (CLP) to induce a polymicrobial sepsis. Sham-operated mice received a similar laparotomy, however without performing the CLP-technique. IAP (New England Biolabs, 1 IU/g of mouse) or vehicle (NaCl 0.9%) were administered 5 minutes prior to the CLP/Sham-procedure and subsequently repeated b.i.d. for the next two days to combine a preventive and curative treatment regimen. Two days after the CLP/Sham-procedure, the abdomen was reopened, 100µl of 4kDa FITC-dextran was injected directly into the ileum and the FITC-dextran serum concentration was measured fluorospectrophotometrically (Excitation 485nm, Emission 518nm) one hour later. Changes in gene expression were measured with RT-PCR. Septic mice lost considerably more weight and had worse clinical disease scores compared to sham groups. Treatment with IAP did not result in improved clinical outcomes. In contrast, following the CLP-procedure, administration of IAP reduced the permeability of the small intestinal barrier for 4kDa FITC-dextran with 50% (Relative serum FITC-Dextran concentration 6.88 ±1.51 for CLP+Vehicle vs. 3.45 ±0.62 p=0.047 for CLP+IAP 1 IU/g mouse). Gene expression of IL1β, IL6, IL10, IL18, TNFα was significantly upregulated following a CLP-procedure. Occludin expression on the other hand was down-regulated. IAP-therapy resulted in a significantly increased expression of Claudin-1 and Claudin-14 in both sham- and CLP-operated mice. In mice, CLP-induced sepsis is associated with disturbed intestinal permeability, modified expression of intestinal tight junctions and increased expression of ileal inflammatory markers. Treatment with IAP partially reversed the sepsis-induced disruption of the intestinal barrier and significantly altered the gene expression of Claudin-1 and Claudin-14. We expect this increase to be linked to the observed improvements of the intestinal barrier function after IAP-treatment. However, despite the observed effects on the intestinal barrier, these improvements did not translate into better clinical outcomes.

P6

TMBIM5, a Bax Inhibitor-1-family member: a novel mitochondrial Ca^{2+}-uptake system?

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The Transmembrane Bax Inhibitor-1 Motif (TMBIM)-containing proteins belong to an evolutionary conserved family that controls cell death processes. To achieve this function, the six TMBIM proteins (TMBIM 1-6), of which TMBIM6 (Bax Inhibitor-1) is the founding member, exploit different mechanisms, including regulation of intracellular Ca^{2+} homeostasis. TMBIM proteins have heterogeneous intracellular localization. Yet, TMBIM5 is the only member localizing in the inner mitochondrial membrane, where it is involved in cristae organization and the release of cytochrome c. Considering the high sequence similarity between TMBIM5 and TMBIM6 and that Ca^{2+}-signaling modulation is a common feature of the TMBIM proteins, we wondered whether TMBIM5 represents a novel transport mechanism of mitochondrial Ca^{2+} across the inner mitochondrial membrane. We used HeLa cells as an overexpression system. We first confirmed the localization of EGFP-TMBIM5 at the mitochondria. Next, we measured the mitochondrial Ca^{2+} uptake in response to ATP by using Rhod-2 AM. Interestingly, we found a significant increase in agonist-induced mitochondrial Ca^{2+} uptake in the TMBIM5-overexpressing cells compared to the control cells. Importantly, the thapsigargin-releasable Ca^{2+} pool and agonist-induced Ca^{2+} release in the cytosol were not altered by TMBIM5 overexpression. This suggests that TMBIM5 may act as Ca^{2+} transporter that mediates the Ca^{2+} flux in the mitochondria. Strikingly, TMBIM5 overexpression rendered HeLa cells more resistant to cell death stimuli like staurosporine and menadione. Further work will focus on TMBIM5's putative pore-forming region near the C-terminus to elucidate its role in mitochondrial Ca^{2+} homeostasis and its contribution to Ca2+-regulated cell death and survival.

P7

Triggering cell death through disruption of the Bcl-2/ip3r interaction in cancer cells: discovering the mechanisms via which BIRD-2 kills diffuse large B-cell lymphoma cells

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The overexpression of B-cell lymphoma 2 (Bcl-2), an anti-apoptotic member of the Bcl-2-protein family, is a common way for cancer cells to avoid apoptosis through cellular stress. Some subtypes of diffuse large B-cell lymphomas (DLBCL) adopt this strategy to circumvent cell death instigated by an upregulation of e.g. Bim or an upregulation of the inositol-1,4,5-trisphosphate (IP3) receptor isoform 2 (IP3R2), a Ca^{2+}-release channel in the endoplasmic reticulum (ER). In the latter case, Bcl-2 represses IP3R2-mediated Ca^{2+} release, by establishing an interaction between its own B-cell homology (BH) 4 domain and the central, regulatory domain of the IP3R2. A novel peptide tool, named Bcl-2/IP3R disrupter-2 (BIRD-2), is able to break the interaction between Bcl-2's BH4 domain and the IP3R, thereby eliciting spontaneous, "toxic" Ca^{2+} signaling and cell death. Thus, while BIRD-2 treatment induces an increase in cytosolic Ca^{2+}, the molecular mechanisms that are triggered upon this cytosolic Ca^{2+} surge and ultimately lead to cell death, remain unknown. Rhod-2-AM measurements revealed that in BIRD-2-sensitive cells (SU-DHL-4), at least part of the Ca^{2+} released in response to BIRD-2 is taken up by the mitochondria, sparking interest in the mitochondria as key players in BIRD-2-induced cell death. Furthermore, we observed opening of the mitochondrial permeability transition pore (mPTP) directly via a Co^{2+}-calcein staining and indirectly through the loss of the mitochondrial membrane potential by using a tetramethylrhodamine (TMRM) staining. In addition, roGFP measurements in our cells indicated reactive oxygen species (ROS) production. Interestingly, both pan-caspase inhibitor ZVAD-FMK and receptor-interacting protein kinase 1 (RIPK1) inhibitor Necrostatin-1 (Nec-1) were able to block cell death partially, indicating that both apoptosis and necroptosis might be activated in response to BIRD-2. Moreover, these events are not seen in DLBCL cells that, despite their overexpression of Bcl-2, are not sensitive to BIRD-2 (e.g. OCI-LY-1). While these experiments uncover part of the mechanism through which BIRD-2 kills DLBCL cells, more work needs to be done to get a clearer and more integrated overview of the molecular events that take place in response to BIRD-2 treatment.

P8

Intramolecular loop/tail interactions involve the SH3 binding domain, controlling Cx43-hemichannel activity

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Interactions between C-terminal tail (CT) and cytoplasmic loop (CL) critically control the activity of Cx43 hemichannels. We previously identified the last 9 amino acids of the CT as a critical determinant for binding the 2nd part of the CL and as an important region for Cx43 hemichannel function. Here, we show that deletion of the last 18 amino acids in the CT only partially lowers the binding to the L2 region, indicating that a second L2-binding region is present in the CT. We propose that the SH3-binding domain present in the CT is a target of L2, since the CTΔSH3Δ18 fails to binding biotin-L2 in surface plasmon resonance (SPR) experiments. Moreover, membrane-permeable SH3 peptide (TAT-SH3) removed the high $[Ca^{2+}]_i$ brake on Cx43-hemichannel activity measured via Ca^{2+} waves and ATP release in HeLa cells exogenously expressing Cx43 as well as in primary bovine corneal endothelial cells. These results were confirmed at the single channel level using whole-cell patch clamp experiments and demonstrated that SH3 peptide acts to promote $[Ca^{2+}]_i$-triggered hemichannel opening. Moreover, TAT-SH3 restored the activity of CT-truncated Cx43 hemichannels ($Cx43^{M242}$) in response to Ca^{2+} ionophore (2 µM, A23187). We also identified the critical role of SH3-binding domain in controlling Cx43-based hemichannels in the full-length Cx43 using 5 mM EGTA or 2 µM A23187-induced ATP-release experiments. We found that ATP release mediated by $Cx43^{\Delta CT18}$ and $Cx43^{\Delta SH3}$-based hemichannels was impaired but not completely abolished. However, $Cx43^{\Delta SH3\Delta CT18}$-based hemichannels completely failed to mediate ATP release in response to EGTA or A23187. Finally, we show that in contrast to activity of $Cx43^{M242}$, the ATP-release properties of $Cx43^{\Delta gap19M242}$-based hemichannels in response to 2 µM A23187 could not be restored by TAT-SH3. This shows that both the CT18 region and the SH3-binding domain in the CT of Cx43 are important for Cx43-hemichannel activity.

P9

Constitutive IP_3 signaling underlies the sensitivity of B-cell cancers to the Bcl-2/IP_3 receptor disruptor BIRD-2

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Diffuse large B-cell lymphoma (DL-BCL) and chronic lymphocytic leukemia (CLL) are characterized by the upregulation of anti-apoptotic Bcl-2 proteins, which interact with the IP_3 receptors (IP_3Rs), thereby suppressing toxic Ca^{2+} release. We engineered a synthetic peptide named Bcl-2/IP3R Disruptor-2 (BIRD-2) that targets the BH4 domain of Bcl-2 and thereby prevents Bcl-2's inhibitory effect on IP_3Rs. We proved that BIRD-2 triggers the death of primary CLL cells and DL-BCL cell lines. Interestingly, DL-BCL cell lines with a high expression of type 2 IP_3R (IP_3R2), the IP_3R isoform with the highest sensitivity to its ligand IP_3, were the most sensitive to BIRD-2, whereas DL-BCL cells expressing low IP_3R2 levels were resistant to the peptide tool. We hypothesized that the high IP_3R2 expression per se is not sufficient for cellular sensitivity to BIRD-2. Rather, the combination of high IP_3R2 expression concomitant with constitutive IP_3 signaling, downstream of the tonically active B-cell receptor, renders DL-BCL cancer cells sensitive to BIRD-2. The basal Ca^{2+} level in SU-DHL-4 cells was significantly elevated due to the constitutive IP_3 production and IP_3-mediated Ca^{2+} release. Under standard growth conditions, this constitutive IP_3 signaling fulfilled a pro-survival role, since inhibition of phospholipase C using U73122 (2.5 µM) caused cell death in SU-DHL-4 cells while milder inhibition of IP_3 signaling with a lower concentration of U73122 (1 µM) or expression of an IP_3 sponge suppressed both BIRD-2-induced Ca^{2+} elevation and apoptosis in SU-DHL-4 cells. Furthermore, we found that in a vast majority (12/14) of primary CLL cells, U73122 treatment suppressed BIRD-2-induced cell death. Taken together, our data indicate that constitutive IP3 signaling in lymphoma and leukemia cells can have dual effects. On the one hand, the constitutive IP_3 signaling is necessary for the survival and proliferation of the cancer cells. However, on the other hand, to survive this chronic signaling the DL-BCL cells are dependent on high Bcl-2 levels to prevent excessive Ca^{2+} release via IP_3Rs. We propose that disrupting the interaction between Bcl-2 and IP_3Rs changes from pro-survival to pro-death the effect of constitutive IP_3 signaling, and pave the way for possible therapeutic approaches.

P10

The functionality of ghrelin-R:D1R interactions in D1R-mediated kindling

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Dopamine receptor signalling influences hippocampal excitability and seizure susceptibility. Dopamine 1-receptor (D1R) activation is proconvulsive and repeated administration of the D1R agonist SKF81297 (5 mg/kg) in mice was shown to gradually induce the development of generalized seizures and a chronic kindled state. The D1R is known to form heterodimeric receptor complexes with other G-protein coupled receptors, notably the ghrelin receptor (ghrelin-R). One protomeric partner in such a receptor complex can alter signalling and trafficking properties of the other. In the particular case of hippocampal ghrelin-R:D1R interaction, canonical D1R-signaling shifts towards ghrelin-R mediated Gαq signalling upon D1R-agonist binding thereby promoting calcium mobilization. We previously demonstrated anticonvulsant effects of ghrelin-R activation in various rodent models. We here investigate whether ghrelin-R modulation via administration of the ghrelin-R agonist macimorelin (5 mg/kg) can affect D1R-mediated kindling established by repeated SKF81297 injections. In preliminary experiments, EEG recordings revealed that macimorelin treated mice (n=3) had an average of three generalized seizures during the total duration of the experiment (five days), while control mice (n=4) had an average of seven (n.s., p=0.20, Mann Whitney U test). Behaviourally, none of the mice receiving macimorelin reached the fully kindled state after five injections, while tonic-clonic seizures were observed in the control group after the 4th or 5th SKF81297 injection. Altogether, the trends in our preliminary data suggest the functional importance of hippocampal ghrelin-R:D1R interactions in excitability, and will be further investigated.