Biogenic Amines
and Related Biologically Active Compounds

Aminy biogenne i pokrewne związki o wysokiej aktywności biologicznej

20-22 October
2022
Lodz, Poland

Invited Speakers:

Tomasz BRZOZOWSKI (Poland)
Nicholas CARRUTHERS (USA)
Jarosław DASTYCH (Poland)
Philippe DE DEURWAERDÈRE (France)
Madeleine ENNIS (UK)
Jerzy JOCHEM (Poland)
Maria Beatrice PASSANI (Italy)
Pertti PANULA (Finland)
Holger STARK (Germany)

XVIII[th] Conference of the Polish Histamine Research Society
XVIII Konferencja Polskiego Towarzystwa Badań Nad Histaminą

Dear Attendees,

Welcome to our conference on biogenic amines and their system constituents that regulate all vital body processes both in health and disease. I am very happy that after a four years break we can meet again face-to-face and not virtually. Fortune is fickle.
It brought to the World a powerful virus and a madman, the events I name π2 (Pi squared). The pandemic has caused approximately 6.6 M deaths and due to Russo-Ukrainian war ca. 80K troops and over 6K civilians have been killed, not to mention the health and economic burden.

As scientists we should put all our efforts to uncover strategies leading to health and peace.

During our meeting, we will have the opportunity to listen to 30 presentations. At this point, I would like to express my sincere thanks to the speakers and all who have contributed to the research. Especially, I am very indebted to our foreign invited speakers who despite the uncertain circumstances left their countries to share with us their knowledge. I believe we will learn a lot in a rather short time. Talks will concern the role of histamine signaling and cytokines storm in Covid-19 infection, activation of different transduction pathways in inflammation and in some other conditions, new potential drug candidates for slowing down neurodegeneration, repurposing of marketed drugs, and other interesting topics.

I wish you a good time here in Lodz, in the conference room and beyond.

Welcome!

Wiesława Agnieszka Fogel

Scientific Programme

Thursday, October 20th 2022

14:00	Arrival, accommodation Ambasador Centrum Hotel, Piłsudskiego 29 St., 90-307 Lodz
16:30 – 18:30	Registration Ambasador Centrum Hotel, Reception Hall
16:30 – 18:30	Poster mounting Conference room “Cypryjska”
18:30	**Welcome Addresses** Conference room “Cypryjska” Prof. Dr. W. Agnieszka Fogel, President of the Polish Histamine Research Society
L1	**MULTIPLE TARGETING AT ADENOSINE A2AR/A1R WITH ADDITIONAL H3R ANTAGONISM FOR THE TREATMENT OF PARKINSON’S DISEASE** Lecture by Holger Stark, *Heinrich Heine University Duesseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Universitaetstr. 1, 40225 Duesseldorf, Germany*
L2	**INTERACTION OF HUMAN MAST CELLS WITH BACTERIOPHAGES** Lecture by Jarosław Dastych, *Laboratory of Cellular Immunology, Institute of Medical Biology of the Polish Academy of Sciences, Lodz, Poland; Proteon Pharmaceuticals S.A., Lodz, Poland*
20:00 – 22:00	Welcome Reception Ambasador Premium Hotel, Restaurant

Friday, October 21st 2022

9:00 – 10:40	**Session I** Chair: Andrzej Pilc, Dorota Łażewska
9:00 – 9:30 **L3**	**ON THE ROLE OF HISTAMINE IN STRIATAL DEVELOPMENT AND PHYSIOLOGY** Pertti Panula *Department of Anatomy, University of Helsinki, Helsinki, Finland*
9:30 – 10:00 **L4**	**THE MAGIC “OF” L-DOPA: FROM PARKINSON’S DISEASE TO NEWBORN RATS** Philippe De Deurwaerdère, Marie Boulain, Zora Pelloquin-Mvogo, Ines Khsime, Rahul Bharatiya, Laurent Juvin, Grégory Barrière *Institute of Cognitive and Integrative Neuroscience of Aquitaine (INCIA); CNRS Bordeaux, France*
10:00 – 10:05 **P1**	**IN VITRO TOXICITY OF (*R*)- AND (*S*)-SALSOLINOL** Gniewomir Latacz[1], Magdalena Kurnik-Łucka[2], Joanna Goryl[1], Nadia Khan[1,2], Mario Rivera[3], Krzysztof Gil[2] *[1]Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland; [2]Department of Pathophysiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland; [3]Laboratory of Experimental Pharmacology, Faculty of Chemical Sciences and Pharmacy, Santiago de Chile, University of Chile*
10:05 – 10:35 **L5**	**HISTAMINE IN HAEMORRHAGIC SHOCK – CENTRAL AND PERIPHERAL PATHWAYS INVOLVED IN CARDIOVASCULAR REGULATION** Jerzy Jochem *Department of Physiology, Medical University of Silesia, Katowice, Poland*
10:35 – 10:40 **P2**	**MODIFIED GOLD NANOPARTICLES AS CARRIERS OF antiAPOE4-siRNA, CHARATERIZATION OF THE COMPLEXES AND THEIR IMPACT ON THE VASCULAR ENDOTHELIUM OF THE BLOOD-BRAIN BARRIER** Piotr Białecki[1], Elżbieta Pędziwiatr-Werbicka[1], Elżbieta Okła[1], Andrea Barrios-Gumiel[2,3,4], Javier Sánchez-Nieves[2,3,4], Rafael Gómez[2,3,4], F. Javier de la Mata[2,3,4], Maria Bryszewska[1] *[1]Department of General Biophysics, Institute of Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland; [2]Dpto. de Química Orgánica y Química Inorgánica, Universidad de Alcalá (UAH), Campus Universitario, Madrid, Spain; [3]Instituto de Investigación Química “Andrés M. del Río” (IQAR), Universidad de Alcalá (UAH), Spain Networking Research Center for Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain; [4]Instituto Ramón y Cajal de Investigación Sanitaria, IRYCIS, Spain*
10:40 – 11:00	Coffee /Tea ***Discussions by Posters***

11:00 – 12:45 **Session II**
Chair: Dariusz Matosiuk, Anna Stasiak

11:00 – 11:30
L6

CHEMOGENETIC CONTROL OF HISTAMINERGIC NEURONS ACTIVITY AFFECTS MALADAPTIVE COGNITIVE RESPONSES TO CHRONIC STRESS IN MICE
Maria Beatrice Passani

Department of Health Sciences University of Florence, Firenze, Italy

11:30 – 11:35
P3

PHENOXYMETHYL DERIVATIVES OF 1,3,5-TRIAZINE AS POTENT SEROTONIN 5-HT$_6$ RECEPTOR LIGANDS
Dorota Łażewska[1], Małgorzata Więcek[1], Michał Pikuła[1], Grzegorz Satała[2], Jadwiga Handzlik[1]

[1]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College in Kraków, Kraków, Poland; [2]Department of Medicinal Chemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

11:35 – 11:55
O1

SEROTONIN: THE STORY ABOUT MOOD AND PROBIOTICS
Michał S. Karbownik[1], Steven D. Hicks[2], Anna Wiktorowska-Owczarek[1], Edward Kowalczyk[1]

[1]Department of Pharmacology and Toxicology, Medical University of Lodz, Łódź, Poland; [2]Division of Academic General Pediatrics, Penn State College of Medicine, Hershey, PA, United States

11:55 – 12:25
L7

HISTAMINE AND COVID-19
Madeleine Ennis

The Wellcome-Wolfson Institute for Experimental Medicine, The Queen's University of Belfast, Belfast, UK

12:25 – 12:45
O2

SARS-CoV-2 PROTEINS INDUCE EXPRESSION OF IL-6. IMPLICATIONS FOR CYTOKINE STORM SYNDROME
Marcin Ratajewski

Laboratory of Epigenetics, Institute of Medical Biology, Lodz, Poland

13:00 – 14:00 Lunch

14:00 – 16:30 **Session III**
Chair: Dariusz Szukiewicz, Barbara Skrzydło-Radomańska

14:00 – 14:25
O3

RAMAN SPECTROSCOPY FOR IDENTIFICATION OF T CELLS ACTIVATION
Aleksandra Borek-Dorosz[1], Paulina Laskowska[2], Anna Maria Nowakowska[1], Patrycja Leszczenko[1], Adriana Adamczyk[1], Małgorzata Zasowska[2], Maciej Szydłowski[2], Małgorzata Baranska[1,3], Katarzyna Maria Marzec[1], Katarzyna Majzner[1,3], Piotr Mrówka[2,4]

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14:25 – 14:45
O4

SIRTUINS PROMOTE BRAIN HOMEOSTASIS, PREVENTING ALZHEIMER'S DISEASE THROUGH TARGETING NEUROINFLAMMATION
Mateusz Wątroba, Dariusz Szukiewicz

Department of Biophysics, Physiology and Pathophysiology, Faculty of Health Sciences, Medical University of Warsaw, Warsaw, Poland

14:45 – 15:05
O5

PROTECTIVE EFFECT OF RESVERATROL ON ASTROCYTE SIRT1 SECRETION (UPON NEUROINFLAMMATION) DEPENDS ON SYSTEMIC GLYCEMIA
Anna D. Grabowska, Mateusz Wątroba, Joanna Witkowska, Agnieszka Mikulska, Dariusz Szukiewicz

Laboratory of the Blood-Brain Barrier, Department of Biophysics, Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland

15:05 – 15:30
O6

THE ROLE OF TNF-α AND ANTI-TNF-α AGENTS DURING PRECONCEPTION, PREGNANCY, AND BREASTFEEDING
Katarzyna Romanowska-Próchnicka[1,2], Piotr Wojdasiewicz[1], Dariusz Szukiewicz[1]

[1]Department of Biophysics, Physiology and Pathophysiology, Faculty of Health Sciences, Warsaw Medical University, Warsaw, Poland; [2]Department of Connective Tissue Diseases, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland

15:30 – 15:50
O7

COMPARATIVE ANALYSIS OF THE OCCURRENCE AND ROLE OF CX3CL1 FRACTALKINE) AND ITS RECEPTOR CX3CR1 IN HEMOPHILIC ARTHROPATHY AND OSTEOARTHRITIS
Piotr Wojdasiewicz[1,2], Łukasz A. Poniatowski[3,4], Andrzej Kotela[5,6], Marta Skoda[1], Michał Pyzlak[1], Aleksandra Stangret[1], Ireneusz Kotela[6], Dariusz Szukiewicz[1]

[1]Department of General and Experimental Pathology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland; [2]Department of Rehabilitation, Eleonora Reicher National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [3]Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland; [4]Department of Neurosurgery, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; [5]Department of Orthopedics and Traumatology, 1st Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland; [6]Department of Orthopedics and Traumatology, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland

15:50 – 16:10
O8

THE ROLE OF KISSPEPTIN-10 IN THE REGULATION OF COLLAGEN METABOLISM IN THE MYOCARDIUM
Paulina Radwańska, Małgorzata Gałdyszyńska, Lucyna Piera, Jacek Drobnik

Department of Pathophysiology, Institute of General and Experimental Pathology, Medical University of Lodz, Lodz, Poland

16:10 – 16:30
O9

ACCUMULATION OF COLLAGEN IS REGULATED BY SUBSTRATE STIFFNESS VIA ACTIVATION OF α2β1 INTEGRIN, FAK AND SRC KINASE IN ATRIAL MYOFIBROBLASTS CULTURES
Małgorzata Gałdyszyńska[1], Radosław Zwoliński[2], Lucyna Piera[1], Jacek Szymanski[3], Ryszard Jaszewski[2], Jacek Drobnik[1]

[1]Department of Pathophysiology, [2]Department of Cardiac Surgery, [3]Research Laboratory CoreLab, Medical University of Lodz, Poland

16:30 – 16:45

Coffee /Tea
Discussions by Posters

16:45

General Assembly of members of the Polish Histamine Research Society, Conference room "Maltańska"

20:00 – 22:00

Dinner with cultural event
Ambasador Centrum Hotel, Restaurant

Dinner with a musical event provided by:
GRZEGORZ SZOSTAK - a bass soloist of the Grand Theatre Lodz and the Grand Theatre - National Opera in Warsaw
and DANUTA ANTOSZEWSKA – a pianist from The Lodz Film School
Ambasador Premium Hotel, Restaurant

Saturday, October 22nd 2022

9:30 – 11:25

Session IV
Chair: W. Agnieszka Fogel, Krzysztof Walczyński

9:30 – 10:00
L8

EXPLOITING THE KINETIC ISOTOPE EFFECT TO IMPROVE THE METABOLIC STABILITY OF OREXIN-1 RECEPTOR ANTAGONISTS
Nicholas I. Carruthers
JANSSEN US (RETIRED)

10:00 – 10:05
P4

FROM BIO-ACTIVITY PROFILING OF GUANIDINES TO THE DISCOVERY OF POTENT MUSCARINIC M_2R/M_4R ANTAGONISTS
Marek Staszewski[1], Dominik Nelic[2], Jakub Jończyk[3], Mariam Dubiel[4], Annika Frank[4], Holger Stark[4], Marek Bajda[3], Jan Jakubik[2], Krzysztof Walczyński[1]

[1]Department of Synthesis and Technology of Drugs, Medical University of Lodz, Łódź, Poland; [2]Department of Neurochemistry, Institute of Physiology CAS, Prague, Czech Republic; [3]Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland; [4]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Duesseldorf, Germany

10:05 – 10:35
O10

NEW MOLECULES AS OPIOID LIGANDS
Dariusz Matosiuk, Dominik Straszak, Tomasz Wróbel, Damian Bartuzi, Agnieszka A. Kaczor

Medical University, Faculty of Pharmacy, Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Lublin, Poland

10:35 – 11:00
O11

ON THE ENHANCEMENT OF ANTIDEPRESSANT-LIKE EFFICACY OF HALLUCINOGENIC DRUGS BY MGLU2/3 RECEPTOR ANTAGONIST OR NEGATIVE ALLOSTERIC MODULATORS (NAMS)
Agnieszka Pałucha-Poniewiera, Bernadeta Szewczyk, Yana Babii, Dorota Bederska- Łojewska, Agata Machaczka, Andrzej Pilc

Institute of Pharmacology, Polish Acad. Sci. Krakow, Poland

11:00 – 11:20
O12

THE IMPACT OF E-98, NOVEL H_3R ANTAGONIST, ON THE PROCESS OF NOCICEPTION AND MORPHINE EFFECTIVENESS IN A NERVE INJURY-INDUCED NEUROPATHIC PAIN MODEL
Katarzyna Popiołek-Barczyk[1], Dorota Łażewska[2], Magdalena Bialon[1], Aleksandra Pędracka[1], Gniewomir Latacz[2], Katarzyna Kieć-Kononowicz[2], Katarzyna Starowicz[1]

[1]Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Neurochemistry, Krakow, Poland; [2]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Krakow, Poland

11:20 – 11:25
P5

REPURPOSING OF POTENTIAL ANTIPSYCHOTICS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES
Agnieszka A. Kaczor[1,2], Oliwia Koszła[1], Przemysław Sołek[3], Ewa Kędzierska[4], Grażyna Biała[4]

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11:25 – 11:30
P6

THE SEARCH FOR CHOLINESTERASE INHIBITORS AMONG HISTAMINE H_3 RECEPTOR LIGANDS
Dorota Łażewska[1], Paula Zaręba[2], Justyna Godyń[2], Tobias Werner[3] Anna Więckowska[2], Barbara Malawska[2], Holger Stark[3], Katarzyna Kieć-Kononowicz[1]

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11:25 – 11:50

Coffe
and Poster discussion

11:50 – 13:20 **Session V**
Chair: Agnieszka Kaczor, Jerzy Jochem

11:50 – 12:10
O13

FUNCTIONAL SELECTIVITY OF NEW HISTAMINE H_4 RECEPTOR LIGANDS FROM THE GROUP OF ALKYL- AND CYCLOALKYL-1,3,5-TRIAZINE DERIVATIVES

Agnieszka Olejarz-Maciej[1], Tadeusz Karcz[1], Monika Głuch-Lutwin[2], Barbara Mordyl[2], Agata Siwek[2], Stefanie Hagenow[3], Jarosław Kupczyk[1], Katarzyna Kamińska[1], Holger Stark[3], Katarzyna Kieć-Kononowicz[1], Dorota Łażewska[1]

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12:10 – 12:30
O14

DIFFERENCES BETWEEN PROTON PUMP INHIBITORS AND HISTAMINE H2 RECEPTOR ANTAGONISTS – ARE THEY REALLY IMPORTANT?

Barbara Skrzydło-Radomańska

Department and Clinic of Gastroenterology, Medical University of Lublin

12:30 – 13:00
L9

DOES THE EXERCISE HAVE AN ALTERNATIVE THERAPEUTIC OPTION IN THE ULCERATIVE COLITIS? ROLE OF SKELETAL MUSCLE AND ADIPOSE TISSUE BIOMARKERS AND INTESTINAL BARRIER PROTEINS

Dagmara Wojcik-Grzybek[1], Aleksandra Danielak[1], Sławomir Kwiecien[1], Marcin Magierowski[1], Zbigniew Sliwowski Z[1], Agnieszka Mazur-Bialy[2], Jan Bilski[2], Tomasz Brzozowski[1]

[1]Department of Physiology, Faculty of Medicine, Jagiellonian University Medical College, Cracow, Poland; [2]Department of Ergonomics and Exercise Physiology, Faculty of Health Sciences, Jagiellonian University Medical College, Cracow, Poland

13:00 – 13:20
O15

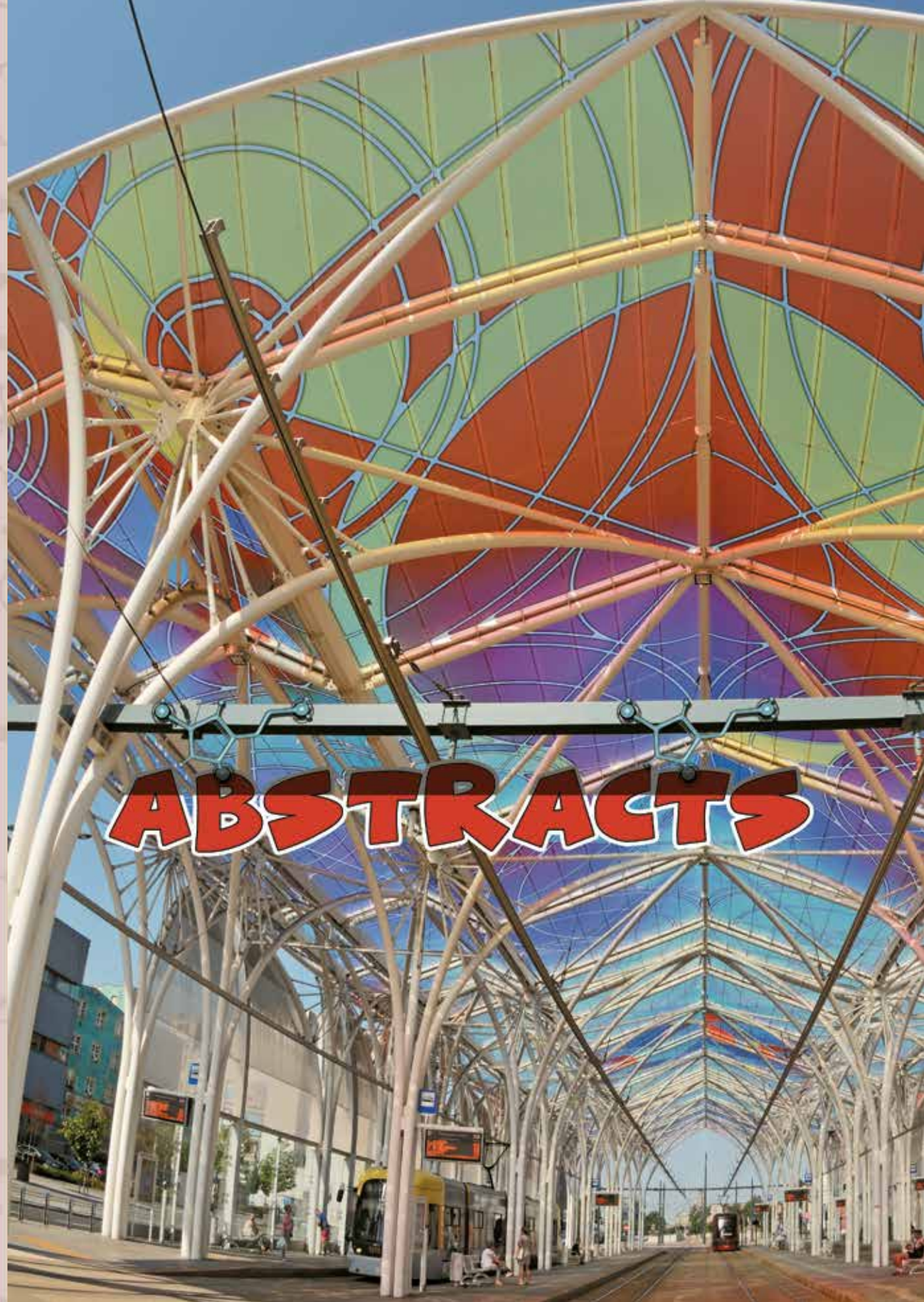
LACTATE OPERATES AS THE HCA1 LIGAND WITH HDAC INHIBITORY ACTIVITY TO MODULATE CELLULAR NHEJ COMPONENTS AND RESTRICT TRANSDUCTION RATE OF RETROVIRAL VECTORS

Waldemar Wagner[1,2], Katarzyna Sobierajska[3], Katarzyna D. Kania[4], Edyta Paradowska[4], Wojciech M. Ciszewski[3]

[1]Department of Hormone Biochemistry, Medical University of Lodz, Łódź, Poland; [2]Laboratory of Cellular Immunology, Institute of Medical Biology PAS, Lodz, Poland; [3]Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland; [4]Laboratory of Virology, Institute of Medical Biology PAS, Lodz, Poland

13:20 Closing Ceremony of the XVIII-th Conference of the Polish Histamine Research Society

13:30 Lunch



Invited Lecture

L1 MULTIPLE TARGETING AT ADENOSINE $A_{2A}R/A_1R$ WITH ADDITIONAL H_3R ANTAGONISM FOR THE TREATMENT OF PARKINSON'S DISEASE

Holger Stark[1], Stefanie Hagenow[1], Anna Affini[1], Elsa Y. Pioli[2], Sonja Hinz [3,4], Yan Zhao[5], Gregory Porras[2], Vigneshwaran Namasivayam[3], Christa E. Müller[3], Jian-Sheng Lin[5], Erwan Bezard[2,6]

[1]Heinrich Heine University Duesseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Universitaetstr. 1, 40225 Duesseldorf, Germany; [2]Motac Neuroscience Ltd., SK10 4TF, Macclesfield, United Kingdom; [3]University of Bonn, PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, An der Immenburg 4, 53121 Bonn, Germany; [4]Center for Biomedical Education and Research (ZBAF), Faculty of Health, Institute of Pharmacology and Toxicology, School of Medicine, University of Witten/ Herdecke, Germany; [5]Laboratory of Integrative Physiology of the Brain Arousal Systems, Lyon Neuroscience Research Center, INSERM UI028, CNRS UMR 5292, Claude Bernard University, 8 Avenue Rockefeller, 69373 Lyon, France; [6]Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, 33000 Bordeaux, France; Correspondence: stark@hhu.de

Adenosine A_1/A_{2A} receptors ($A_1R/A_{2A}R$) represent targets in non-dopaminergic treatment of motor disorders like Parkinson's disease (PD). As innovative strategy, multi-targeting ligands (MTLs) were developed to achieve comprehensive PD therapies simultaneously addressing comorbid symptoms like sleep disruption. Recognizing the wake-promoting capacity of histamine H_3 receptor (H_3R) antagonists in combination with the "caffeine-like effects" of $A_1R/A_{2A}R$ antagonists, we designed $A_1R/A_{2A}R/H_3R$ MTLs, where a piperidino-/ pyrrolidino(propyloxy)phenyl H_3R pharmacophore was introduced into an adenosine antagonist arylindenopyrimidine core [1]. These MTLs showed distinct receptor binding profiles with overall nanomolar H_3R affinities (K_i < 55 nM).

Compounds 4 (4-phenyl-8-(3-(pyrrolidin-1-yl)propoxy)-5*H*-indeno[1,2-*d*]pyrimidin-2-amine, ST-2001: $K_i(A_1R)$ = 11.5 nM, K_i ($A_{2A}R$) = 7.25 nM) and 12 (2-amino-8-(3-(piperidin-1-yl) propoxy)-4-phenyl-5*H*-indeno[1,2-*d*]pyrimidin-5-one, ST-1992: K_i (A_1R) = 11.2 nM, K_i ($A_{2A}R$) = 4.01 nM) were evaluated *in vivo* in rodents. L-DOPA-induced dyskinesia was improved after administration of compound 4 (1 mg/kg, i.p. rats). Compound 12 (2 mg/kg, p.o. mice) increased wakefulness representing a novel pharmacological tools for PD therapy.

References

1. Hagenow S, Affini A, Pioli EY, et al. Adenosine A2AR/A1R Antagonists Enabling Additional H_3R Antagonism for the Treatment of Parkinson's Disease. J Med Chem. 2021; 64 (12): 8246-8262.

Invited Lecture

L2 INTERACTION OF HUMAN MAST CELLS WITH BACTERIOPHAGES

Aurelia Walczak-Drzewiecka[1], Michał Różański[1], Marta Zygmunt[2], Ewelina Wójcik[2], Jarosław Dastych[1,2]

[1]Laboratory of Cellular Immunology, Institute of Medical Biology of the Polish Academy of Sciences, Lodz, Poland; [2]Proteon Pharmaceuticals S.A., Lodz, Poland

Aim. Bacteriophages are bacterial viruses controlling number of bacteria in the biosphere that constitute significant part of human microbiome. These nanoparticles are known to be internalized by eukaryotic cells. We investigated hypothesis that bacteriophages are internalized by mast cells, stored in mast cell granules and released as active viral particles capable to infect bacterial cells.

Materials and Methods. Human mast cell line LAD2 was cultured under standard condition in cell line specific media. The source of S. aureus and S. aureus specific phages was Proteon Pharmaceuticals (PP) collection of bacterial strains and bacteriophages. LAD2 mast cells were incubated with different numbers of S. aureus and live/dead cells were analyzed after propidium iodide staining by FACS analysis using BD LSR Fortessa flow cytometer. Number of live bacteria in suspensions were determined by a traditional plate method. Enumeration of bacteriophages in a suspension was performed by traditional double agar overlay plaque assay. Gene expression was analyzed by real time RT-PCR in a LightCycler 480 (Roche) using SybrGreen I after synthesis of complementary DNA (cDNA) from total RNA.

Results. First, we determined the effect of live S. aureus bacteria on viability of human mast cells. When LAD2 mast cells were incubated with different numbers of live S. aureus we observed massive death and fragmentation of human mast cells. Preincubation of mast cells with bacteriophages, followed by extensive washing by suspension resulted in significant protection of these cells from death and fragmentation occurring following exposure to live S. aureus. Incubation of mast cells with bacteriophage suspension followed by extensive washing and lysis in deionized water showed the presence of significant number of phages in cell lysates. Control mock lysate (without cells) contained virtually no phages. When LAD2 mast cells were incubated with S. aureus and bacteriophages for 20 h. and gene expression analyzed by real-time RT-PCR we observed in mast cells exposed to S. aureus induction of gene expression of proinflammatory cytokines IL-13, TNF and chemokine CCL3. We did not observed upregulation of these genes upon exposure of mast cells to S. aureus together with specific bacteriophages. Interestingly bacteriophages alone upregulated expression of cytokine gene IL-1beta, chemokine gene CCL5, and integrin coding gene ITGAV.

Conclusions. Series of experimental observations in vitro, demonstrated that pretreatment of human mast cells with bacteriophages specific for pathogenic bacteria resulted in protection of these cells from cytotoxicity mediated by this pathogenic bacterium and that bacteriophages are preferentially associated with cell pellet. These observations support hypothesis that bacteriophages internalized by mast cells are stored in mast cells and released as active viral particles capable to infect bacterial cells.

References

1. Weitz JS, Wilhelm SW. Ocean viruses and their effects on microbial communities and biogeochemical cycles. F1000 Biol Rep. 2012.

2. Bichet MC, et al. Bacteriophage uptake by mammalian cell layers represents a potential sink that may impact phage therapy. iScience. 2021.

3. Rocha-de-Souza CM et al. Human mast cell activation by Staphylococcus aureus: interleukin-8 and tumor necrosis factor alpha release and the role of Toll-like receptor 2 and CD48 molecules. Infect Immun. 2008.

Invited Lecture

L3 ON THE ROLE OF HISTAMINE IN STRIATAL DEVELOPMENT AND PHYSIOLOGY

Pertti Panula

Department of Anatomy, University of Helsinki

Hypothalamic histaminergic neurons regulate a variety of homeostatic, metabolic and cognitive functions. Recent data have suggested a modulatory role of histamine and histamine receptors in shaping striatal activity and connected the histaminergic system to neuropsychiatric disorders. We characterized exploratory behavior and striatal neurotransmission in mice lacking the histamine producing enzyme histidine decarboxylase (Hdc). The mutant $Hdc^{-/-}$ mice showed a distinct behavioral pattern during exploration of novel environment, specifically, increased frequency of rearing seated against the wall, jumping and head/body shakes. This behavioral phenotype was associated with decreased levels of striatal dopamine and serotonin and increased level of dopamine metabolite DOPAC. Gene expression levels of dynorphin and enkephalin, opioids released by medium spiny neuro ns of striatal direct and indirect pathways respectively, were lower in Hdc mutant mice than in control animals. A low dose of amphetamine led to similar behavioral and biochemical outcomes in both genotypes. Increased striatal dopamine turnover was observed in Hdc KO mice after treatment with dopamine precursor L-Dopa. Overall, our study suggests a role for striatal dopamine and opioid peptides in formation of distinct behavioral phenotype of Hdc KO mice. These results are in agreement with our data on morphological characterization of the striatal and cortical neuron systems in $Hdc^{-/-}$ mice, which suggest that no clear abnormalities in comparison with wild-type animals can be detected. Further studies showed that histamine interacts with dopamine in striatal neurons through receptor heterodimers. Histamine may thus be responsible for regulating motor functions as a one of modulators of excitability rather than a developmental factor.

L4 THE MAGIC "OF" L-DOPA: FROM PARKINSON'S DISEASE TO NEWBORN RATS

Philippe De Deurwaerdère, Marie Boulain, Zora Pelloquin-Mvogo, Ines Khsime, Rahul Bharatiya, Laurent Juvin, Grégory Barrière

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Mercuri and Bernardi published in 2006 a commentary article entitled "the magic L-DOPA" where they recalled and addressed the superiority of exogenous L-DOPA, the metabolic precursor of dopamine (DA), over the dopaminergic agonists in the treatment of Parkinson's disease. Fifteen years later, the superiority of L-DOPA is still a magical affair, but the lines are moving. It is no longer sure that DA in the striatum plays a pivotal role in the therapeutic benefit of L-DOPA. Indeed, L-DOPA-derived DA release occurs in parkinson's models via the unique involvement of serotonin (5-HT) neurons which are widespread in the central nervous system (CNS). Interestingly, while the destruction of ascending 5-HT neurons suppressed L-DOPA-induced dyskinesia in rodents, it did not alter L-DOPA motor response. L-DOPA has been shown for years to be unique in triggering in mammals "magical", locomotor responses presumably by acting on spinal cord centres. In this presentation, we will briefly enumerate the experimental arguments leading to leave the striatum as a main target of L-DOPA-derived DA, and present data concerning the pro-locomotor effects of L-DOPA in the air stepping model in the newborn rats, an experimental model that we have re-investigated.

The air stepping model has been developed to study locomotor pattern in newborn rats in a situation where the proprioceptive feedbacks are minimized. We confirmed that L-DOPA (25, 50, 75, 100 mg/kg) triggers a spectacular, dose-dependent locomotor response at P5, allowing us to precisely recognize fundamental criteria of locomotor patterns using a kinematic analysis. A tissue evaluation of the neurochemical effects of L-DOPA indicated that L-DOPA dramatically enhanced DA tissue content in the spinal cord, as well as its metabolites without altering noradrenaline and 5-HT tissue content. In the brain, the neurochemical responses were diverse, the mesencephalic locomotor region, the M1 cortex and the nucleus accumbens responding slightly more than other regions. Conversely, the exogenous administration of the 5-HT precursor 5-hydroxytryptophan did not trigger locomotor episodes, but opposed the prolocomotor and spinal cord neurochemical effects of L-DOPA, presumably by competing with L-DOPA on the aromatic amino acid decarboxylase. Moreover, the use of the catechol-*O*-methyl transferase inhibitor tolcapone, or the non-selective monoamine oxidase inhibitor nialamide boosted the effect of L-DOPA on some locomotor responses and the spinal cord biochemical effects.

In conclusion, the magic in L-DOPA's locomotor responses could involve DA in spinal cord in neonates, with some adjustments performed in supraspinal regions. The extent to which such a mechanism could be involved in the treatment of Parkinson's disease remains to be explored.

15 HISTAMINE IN HAEMORRHAGIC SHOCK - CENTRAL AND PERIPHERAL PATHWAYS INVOLVED IN CARDIOVASCULAR REGULATION

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Acute haemorrhage leads to two phases of haemodynamic and neurohormonal response – an initial reflex-induced increase in the sympathetic system activity (sympathoexcitatory phase) followed by a decrease in the sympathetic activity (sympathoinhibitory phase). Peripheral tissue hypoperfusion in haemorrhagic shock and subsequent reperfusion evoke immunological changes which may lead to multiorgan dysfunction/systemic inflammatory response syndrome.

Histamine is able to affect both central and peripheral mechanisms of cardiovascular regulation in haemorrhagic shock. Experimental studies show the activation of the central histaminergic system in haemorrhagic hypotension. In these conditions, centrally acting histamine is able to induce a reversal of critical haemorrhagic hypotension due to the activation of the sympathetic and the renin-angiotensin systems as well as increases in vasopressin and melanocortin peptides secretion.

Histamine H_3 receptors are present on postganglionic endings of the sympathetic neurons and regulate the synthesis and release of postganglionic neurotransmitters. Activation of H_3 receptors located on postganglionic endings innervating vessels leads to a vasodilatation. The blockage of H_3 receptors at the sympathoinhibitory phase of haemorrhagic shock induces a long-lasting increases in blood pressure and peripheral blood flows. The effects are inhibited by peripheral chemical sympathectomy with 6-hydroxydopamine and intravenous pre-treatment with α_1-adrenoceptor antagonist prazosin, but not with β-adrenoceptor blocker propranolol. In addition, the pressor effect is accompanied by reduced increases in plasma proinflammatory cytokines, such as TNF-alpha, IL-1alpha, IL-1beta, IL-12 and IFN-alpha.

In conclusion, histamine acting centrally and peripherally is able to influence the cardiovascular system function in haemorrhagic shock and to modulate hypoxia/reperfusion-induced immunological changes in critical hypovolaemia.

16 CHEMOGENETIC CONTROL OF HISTAMINERGIC NEURONS ACTIVITY AFFECTS MALADAPTIVE COGNITIVE RESPONSES TO CHRONIC STRESS IN MICE

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The histaminergic system has a cardinal role in regulating animals' performance in various learning paradigms and feeding behaviour. However, our current knowledge of the role of neuronal HA in memory and feeding behaviour is based on its depletion, or acute local injections of histaminergic ligands, which present inherent technical limitations.
Aim: Here, we investigated the impact of activating or silencing endogenous histaminergic neurotransmission using chemogenetic tools.

Methods. To interrogate the function of brain HA we used the DREADDs-driven technology injecting HDC-Cre mice bilaterally into the TMN with excitatory or inhibitory DREADDs to transfect histaminergic cells. The mice were then tested for social and fear memories, and feeding behaviour.

Results. We observed an opposite effect when TMN^{HA} cells are modulated. When stimulated the HA neurons improve social and fear memories, whereas when inhibited an impairment is apparent. Consistently, TMN^{HA} stimulation induces a reduction of food intake. TMN^{HA} inhibition, instead, is responsible for increased food intake.

Conclusions. We revealed that selective chemogenetic activation or inhibition of TMN^{HA} cells results in facilitation or impairment of memory, respectively. Similarly, HA cells activation reduces, whereas its inhibition increases food intake. These results confirm and expand previous reports regarding the role of neuronal HA in the regulation of memory and feeding behaviour and pave the way for future studies to deconstruct specific histaminergic neural pathways involved in different types and memory phases as well as the regulatory mechanisms underlying the control of feeding.

17 HISTAMINE AND COVID-19

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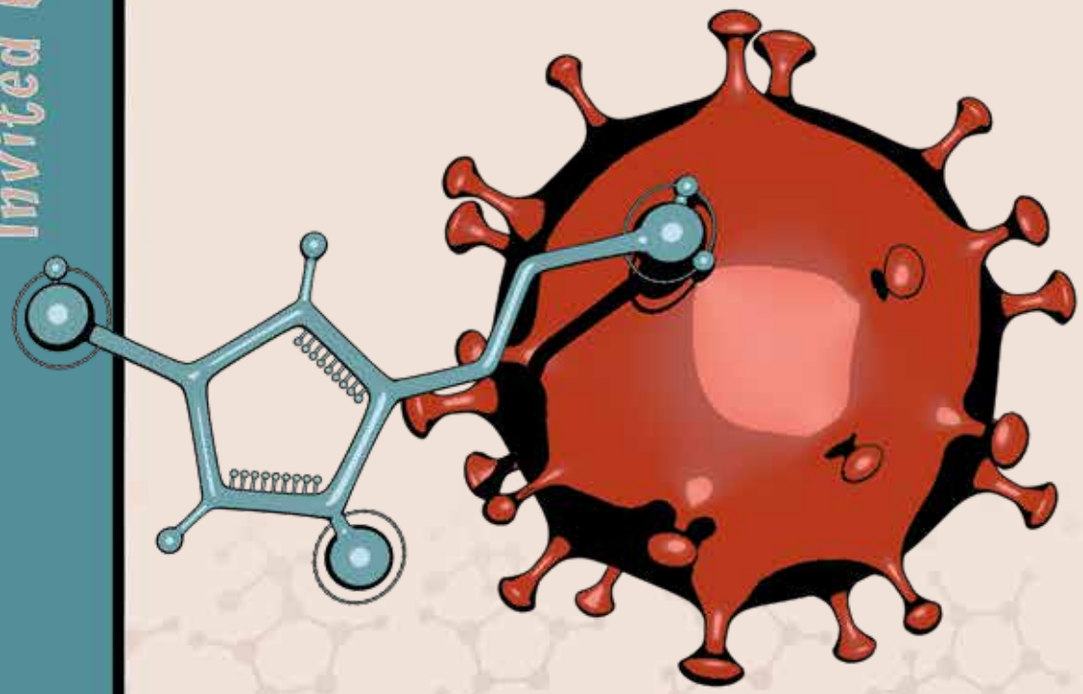
In April 2020, reports in the popular press suggested that famotidine could act as a treatment of COVID-19. Unpublished, non-significant data (death rate in patients taking famotidine half that in those not taking the drug) led to further studies. This talk will describe clinical studies investigating the effect of histamine antagonists in patients with COVID-19. Given the time to develop new drugs, much research has concentrated on drug repurposing. Among a great many other drugs, H_1 and H_2 antagonists, as well as cromolyn, a 5-Lipoxygenase inhibitor and leukotriene receptor antagonist have been identified as potential treatments. This presentation will examine the available evidence for histamine antagonists as treatments for COVID-19, the potential effects of histamine antagonists on the virus and will explore the immunomodulatory actions of histamine via the H_2 receptor, which may play a role in the progression of COVID-19.



18 EXPLOITING THE KINETIC ISOTOPE EFFECT TO IMPROVE THE METABOLIC STABILITY OF OREXIN-1 RECEPTOR ANTAGONISTS

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JANSSEN US (Retired)

The orexin system consists of two neuropeptides orexin-A and orexin-B that originate in neurons situated within the hypothalamus and play important roles in sleep/wake cycles, circadian rhythms, energy/metabolism, reward directed behaviors, stress responses and monoamine release via a discrete network of neuroanatomical projections. They exert their effect via stimulating two G-protein coupled receptors, orexin-1 (OX_1R) and orexin-2 (OX_2R) respectively that are collocated or selectively located in specific brain regions implying differentiated roles. The anatomical distribution of OX_1R is consistent with a role in panic or anxiety states. Optimizing a substituted azabicylo[2.2.1]heptane dual receptor antagonist, which was initially prepared as an exploratory series for an orexin-2 program, identified the selective OX_1R antagonist JNJ-54717793 as a candidate for clinical development [1,2].

This presentation will focus on reducing the efflux potential and improving the brain penetration of JNJ-54717793 while maintaining low clearance. In order to achieve the latter objective, we exploited the kinetic isotope effect to reduce in vivo clearance which ultimately afforded JNJ-61393215 which has completed Phase I clinical trials.

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DOES THE EXERCISE HAVE AN ALTERNATIVE THERAPEUTIC OPTION IN THE ULCERATIVE COLITIS? ROLE OF SKELETAL MUSCLE AND ADIPOSE TISSUE BIOMARKERS AND INTESTINAL BARRIER PROTEINS

L9

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Background. The role of exercise in obese patients with inflammatory bowel disease (IBD) remains largely unknown. Human IBD, consisting of Crohn's disease and ulcerative colitis (UC), is resistant to treatment and is characterized by an alternating cycle of active and resting states that significantly worsen the physical function and quality of life of patients. Since pharmacotherapy of IBD often causes adverse effects in humans, alternative therapies that may be of benefit should be tested in experimental models for these diseases.

Aims. We determined whether voluntary and forced exercise with or without treatment with intestinal alkaline phosphatase (IAP), an enzyme known to maintain intestinal tight junction connections and barrier integrity, could influence experimental colitis in exercise versus sedentary obese mice.

Materials and Methods. Two major series of C57BL/6 male mice were fed *ad libitum* high fat diet (HFD, 70% energy from fat, series A) or standard diet (SD,10% energy from fat, series B) (Altromin, Lage, Germany) for 12 weeks and subjected to voluntary on spinning wheels or forced treadmill exercise (15 min/day, 6 wks., Panlab, Harvard Apparatus, Boston, MA, USA) with or without the intragastric treatment with IAP (200 U/day) and trinitrobenzene sulfonic acid (TNBS) colitis was induced. Following colitis, the colonic blood flow (CBF) was examined by Laser Doppler flowmetry, the gross and microscopic injury of colonic mucosa, the caloric intake and muscle strength (BIO-GS3 device, Canada) were assessed. The parameters of oxidative stress markers MDA+4-HNE, GSH+GSSG contents and SOD activity and the colonic expression of proinflammatory biomarkers IL-1β, TNF-α, MCP-1and IL-12 mRNAs, and the tight junctions protein occludin, ZO-1, and adipomyokine irisin were evaluated by qPCR and Western blot, respectively. Proinflammatory markers in plasma: IL-2, IL-6, IL-10, IL-12p70, IL-17, TNF-α, MCP-1 and leptin was performed using Luminex microbeads fluorescent assays.

Results. In sedentary SD mice the DAI was accompanied by a significant fall in CBF and muscle strength and these changes were exacerbated in obese mice. Voluntary effort significantly reduced the DAI but treadmill exercise exacerbated DAI activity in obese mice and decreased CBF vs. SD fed mice ($P<0.05$) and significantly increased the MDA+4-HNE, GSH+GSSG contents, SOD activity, the mRNAs expression of IL-1β, TNF-α, MCP-1, IL-12 mRNAs and protein for phospho-NFκB, MMP-9 and iNOS ($P<0.05$). In obese mice, the downregulation of occludin, ZO-1 and TIMP-1 protein expression was observed. Treatment with IAP decreased DAI in force exercising mice, improved relative muscle strength and significantly reduced the mRNA expression of IL-1β, TNF-α, MCP-1, IL-12 ($P<0.05$) and downregulated phospho-NFκB, MMP-9 protein while increasing protein expression of occludin and ZO-1 and these effects were significantly more pronounced in mice fed HFD vs. SD. Conclusions. Voluntary exercise accelerated the healing of colitis, while forced treadmill exercise exaggerated the severity of colon damage in obese mice through a mechanism involving a decrease in colonic microcirculation due to oxidative stress in the colonic mucosa and an increase in local and systemic pro-inflammatory biomarkers. IAP counteracted the intensifying influence of forced effort on the course of experimental colitis by reducing oxidative stress, lowering the level of pro-inflammatory biomarkers and increasing the expression of tight junction proteins in the colon mucosa.

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IN VITRO TOXICITY OF (R)- AND (S)-SALSOLINOL

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Background and Aim. salsolinol (SAL), a tetrahydroisoquinolines representative, was found in areas with increased dopamine synthesis and turnover such as the ventral midbrain and striatum. SAL was also found in the urine of Parkinson's disease (PD) patients treated with levodopa, and its lower levels were found in the caudate nuclei of PD in comparison with normal human brain. SAL has an asymmetric centre at C1, and both enantiomers, yet, with a predominance of (*R*)-SAL, were detected in human and rat brain tissue. Still, it remains unclear, if racemic SAL and its enantiomers have either neurotoxic or neuroprotective properties. Thus, the aim of the present study was to purify SAL enantiomers from commercially available racemic mixture and to assess their toxicity in neuroblastoma SH-SY5Y cell line.

Material and Methods. (*R,S*)-SAL was purified by means of HPLC with retention time 17.058 min and 21.575 min for (*S*)-SAL and R-SAL, respectively. SH-SY5Y cells were seeded at a concentration of 2.5×10^4 cells/well and cultured for 24 h to reach 70% confluence. Cells were preincubated for 1 h with either racemic SAL and its enantiomers, and followed by incubation with MPP^+ (1000 μM). After 24-48 h of incubation, the MTS assay was used to assess cell viability. Furthermore, the cells were stained with Hoechst 33258 and rhodamine 123 and visualized by using of Leica DMi8 fluorescent microscopy.

Results. The eluates contained (*S*)-SAL with less than 0.1% of (*R*)-SAL as well as (*R*)-SAL with about 4% of (*S*)-SAL, were aliquoted, lyophilized, and stored in dark microtubes. The amount of the purified SAL enantiomers was further checked spectrophotometrically. Cell viability was significantly increased in SH-SY5Y cells exposed to racemic SAL and its enantiomers (all at the concentration of 50 μM) co-incubated with MPP^+ (1000 μM) in comparison to MPP^+ alone.

Conclusions. Our results confirms that neither racemic SAL nor its enantiomers are neurotoxic at low doses in neuroblastoma SH-SY5Y cell line. Moreover, the neuroprotective effect of both enantiomers of SAL were shown against MPP^+ toxic activity. Finally, no statistical differences between neuroprotective potential of SAL enantiomers and racemate were observed.

MODIFIED GOLD NANOPARTICLES AS CARRIERS OF ANTIAPOE4-SIRNA, CHARATERIZATION OF THE COMPLEXES AND THEIR IMPACT ON THE VASCULAR ENDOTHELIUM OF THE BLOOD-BRAIN BARRIER

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The blood-brain barrier is the physical and biochemical barrier between blood vessels and nervous tissue. Its task is to protect the nervous system against harmful factors and to enable selective transport of substances from the blood to the cerebrospinal fluid. On the other hand, the presence of a barrier is an obstacle to the supply of drugs used in neurodegenerative diseases, e.g. Alzheimer's disease.

Aim. The aim of the research: to investigate the interactions of PEGylated, gold nanoparticles stabilized with carbosilane dendrons with siRNA1 and siRNA2 directed against the APOE4 gene planned for anti-Alzheimer's disease therapy, to study their stability and to evaluate the toxicity of free nanoparticles and complexed with antiAPOE4-siRNA.

Materials: two kinds of bifunctionalized AuNPs with two ligands containing a thiol moiety: the cationic dendrons $HSG_2(S\text{-}NMe^{3+})_4$ and a commercial (polyethylene) glycol (PEG) ligand $(CH_3O(CH_2CH_2O)nCH_2CH_2SH$, HS-PEG, Mn = 800) with dendron/PEG ratios 3/1 AuNP14a and 1/1 – AuNP14b [1]; two kinds of antiAPOE4 siRNAs; HBEC-5i cell line, erythrocytes. Methods: gel electrophoresis (also performed in the presence of ribonuclease and heparin) to study of interactions nanoparticle-siRNA and stability of complexes; laser Doppler electrophoresis and dynamic light scattering to study zeta potential and hydrodynamic diameter of complexes; MTT and LDH assay to study the cytotoxicity; haemolysis test to study haemotoxicity.

Results. PEGylated gold nanoparticles stabilized with carbosilane dendrons have the ability to form complexes with antiAPOE4-siRNAs and protect antiAPOE4-siRNA against the ribonuclease activity. Moreover, they have a positive zeta potential at a concentration where complete complexation with antiAPOE4-siRNA takes place. They also form complexes/agglomerates with a hydrodynamic diameter in the range 190 - 2000 nm (dependent on type and concentration of AuNPs). AuNP14a and AuNP14b – siRNAs complexes have lower haemolytic properties than uncomplexed gold nanoparticles and no lower cytotoxicity of complexes was observed compared to uncomplexed gold nanoparticles.

Conclusion. The conducted research gives reasons to qualify PEGylated gold nano particlesstabilized with carbosilane dendrons as a potential carriers of siRNAs directed against theAPOE4 gene planned for Alzheimer's disease therapy. Based on the conducted preliminary studies, further stages of in vitro and in vivo tests are necessary to assess the ability of nanoparticles to cross the blood-brain barrier.
The research was supported by the project NanoTENDO financed by the National Science Centre, Poland under the M-ERA.NET 2, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 685451.

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PHENOXYMETHYL DERIVATIVES OF 1,3,5-TRIAZINE AS POTENT SEROTONIN 5-HT$_6$ RECEPTOR LIGANDS

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Background. The serotonin 5-HT$_6$ receptor (5-HT$_6$R) is located in brain areas involved in learning and memory processes. Intensive preclinical studies have shown that 5-HT$_6$R antagonists could be a promising drug with cognitive improvement in psychiatric (e.g. schizophrenia, depression) or neurodegenerative diseases (e.g. Alzheimer's disease) [1,2]. Our group found potent and selective 5-HT$_6$R agents with 1,3,5-triazine moiety [3]. Among them very promising was [4-((2-isopropyl-5-methylphenoxy)methyl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine (MST4) with high affinity for 5-HT$_6$R (K_i = 11 nM) [4].

Aim. Synthesis and biological evaluation of analogues of our lead structure MST4.

Materials and Methods. Two series of compounds (*mono-* and *di-substituted* derivatives) were obtained by a cyclic condensation of proper methyl esters with 4-methylpiperazin-1-yl biguanide dihydrochloride as described previously [3]. Next, all compounds were evaluated for their affinity for 5-HT$_6$R using [^{3}H]-LSD as a radioligand in HEK293 cells stably transfected with full-length of the 5-HT$_6$R. Selectivity towards other serotonin receptors (5-HT$_{2A}$ and 5-HT$_7$) was checked also in the radioligand binding assays using [^{3}H]-ketanserin (5-HT$_{2A}$) and [^{3}H]-raclopride (5-HT$_7$) as radioligands.

Results. All tested compounds exhibited affinities for 5-HT$_6$R in a nanomolar range (6 nM ≤ K_i ≤ 825 nM). Compounds in the "*mono-substituted*" series showed higher affinities for 5-HT$_6$R than "*di-substituted*" compounds. A few compounds showed also low micromolar affinity for 5-HT$_{2A}$ receptor.

Conclusions. Introduced structural modifications to MST4 let us obtain potent 5-HT$_6$R ligands. The most promising compound, TR-DL-34 ([4-((2-*tert*-butyl)phenoxy)methyl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine]; 5-HT$_6$R: K_i =13 nM; 5-HT$_{2A}$R: K_i = 2183 nM; 5-HT$_7$R: K_i = 6336 nM), was selected for further *in vitro* studies.

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SEROTONIN: THE STORY ABOUT MOOD AND PROBIOTICS

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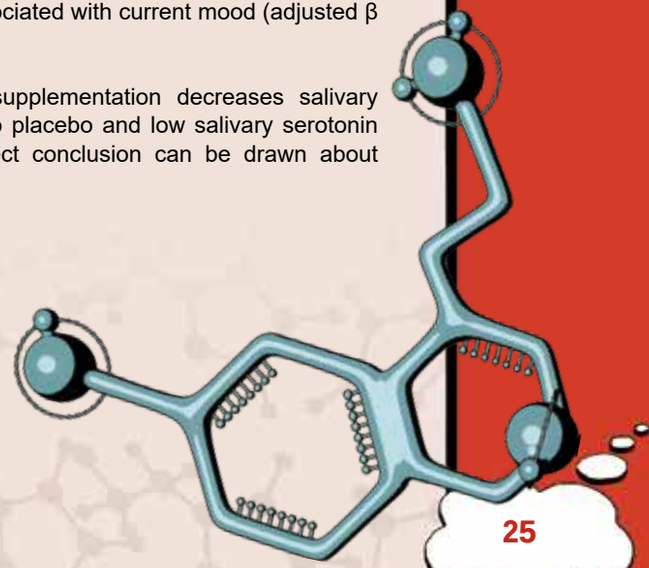
Serotonin plays a pivotal role in numerous biological processes, including the central nervous system functioning. Although serotonin drop was originally linked with depressed mood, the recent research did not fully confirm that initial suggestions. Also, in the light of recent advances in understanding of the gut-brain axis, serotonin was proposed to mediate an antidepressant effect of probiotics. On the other hand, the biology of serotonin is too complex to allow for simple generalizations, and not all specific probiotics have been tested.

Aim. The aim of the present study was to evaluate the effect of a fungal probiotic strain *Saccharomyces boulardii* CNCM I-1079 on salivary serotonin concentration in healthy adults under psychological stress and to link serotonin level in saliva with current mood of another sample of young, physically active people.

Materials and Methods. A randomized placebo-controlled trial was conducted to test 30-day supplementation of *Saccharomyces boulardii* CNCM I-1079 in a dose of 1 billion colony forming units a day. Serotonin concentration was determined with enzyme-linked immunosorbent assay in salivary samples obtained before supplementation and at its end – a day before a stressful event. In addition, saliva was collected before and after training of a group of athletes and serotonin level was assessed with the same method. Current mood was evaluated with the pictogram-enhanced visual analog mood scale.

Results. *Saccharomyces boulardii* supplementation was found to reduce salivary serotonin level by 3.1 (95%CI 0.2 to 6.1) ng/mL, p=0.037, as compared to placebo. In a separate group of people, salivary serotonin level was negatively associated with current mood (adjusted β = −0.32, 95%CI −0.62 to −0.02, p=0.037).

Conclusions. *Saccharomyces boulardii* probiotic supplementation decreases salivary serotonin concentration under stress as compared to placebo and low salivary serotonin is linked to better current mood. However, no direct conclusion can be drawn about antidepressant effect of *Saccharomyces boulardii*.



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SARS-COV-2 PROTEINS INDUCE EXPRESSION OF IL-6. IMPLICATIONS FOR CYTOKINE STORM SYNDROME

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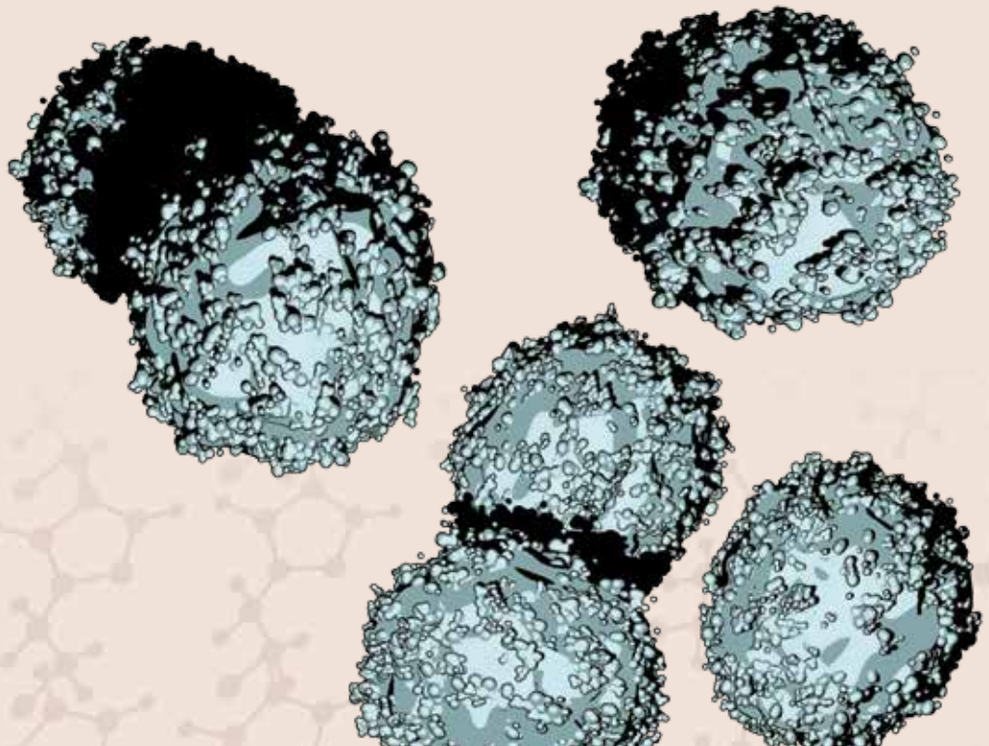
The outbreak of the SARS-CoV-2 virus in December 2019 has caused the deaths of several million people worldwide. During the course of COVID-19 infection most patients have mild symptoms, unfortunately others experience rapid deterioration, which might be associated with systemic inflammation and cytokine storm syndrome. A cytokine storm is caused by immune imbalance and self-perpetuating inflammatory reactions that leads to acute respiratory distress syndrome (ARDS), which is the main cause of death of patients infected with SARS-CoV-2. Among cytokines that have been associated with the ARDS caused by coronavirus infections, IL-6 is particularly important in the persistence of the proinflammatory milieu. Here, using numerous methods (Human Inflammation Array, Real time RT-PCR and ELISA) we show that SARS-CoV-2 viral proteins (nucleocapsid and spike) induce the expression of IL-6 and other proinflammatory cytokines, suggesting that these cells may be the initial source of IL-6 and participate in the development of cytokine storms during COVID-19 infection. Further study identified that chlorpromazine inhibits the expression and secretion of IL-6 by monocytes activated by SARS-CoV-2 virus nucleocapsid protein and affects the activity of NF-κB and MEK/ERK signaling. Thus, we believe that chlorpromazine which is clinically approved neuroleptic might be a desirable drug to support the therapy of patients with COVID-19.



RAMAN SPECTROSCOPY FOR IDENTIFICATION OF T CELLS ACTIVATION

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The immune system responds to various types of threats by increasing the level of readiness and activating the appropriate effectors. Lymphocytes are responsible for the most advanced part of this system, a long-lasting, highly specific adaptive response. T cells are activated upon contact with the antigen presented by APC (antigen presenting cells), but the costimulatory and immunomodulatory effects of other surface proteins and cytokines, including histamine is necessary for fully operational state [1, 2]. Activating signal triggers a series of biochemical intracellular events leading to production of specific effector proteins and accelerated proliferation [3], thus activation of T cells can serve as indicator of an ongoing inflammatory process engaging adaptive response. Current cytometric methods of analysis of blood cells are focused on surface markers recognized by monoclonal antibody. That strategy limit the possible utilization of sorted this way cells in immunotherapy such as CAR-T. Raman spectroscopy imaging, a method based on Raman scattering of light my help to overcome this disadvantage.

Aim. The aim of the study was to determine the specific Raman spectra features that allow the recognition of activated state of T cells.

Materials and Methods. We used blood from healthy donors, isolated T cells using EasySep system and activated cells using microbeads. Immunophenotyping, confocal microscopy and biochemical methods were used to verify activation. We applied a label-free Raman spectroscopy imaging (WITEC) for molecular characterization and discrimination of naïve and activated T cells.

Results. We have defined spectra biomarkers detected and visualised on the sub-cellular level characteristic for carotenoids, nucleic acids as well as proteins and lipids fractions for this purpose. Detailed analysis of the average spectra and PCA and PLS computational methods were applied. We found that carotenoids accumulate in naïve T cells and decrease upon activation. We also observed also accumulation of lipid droplets increased nucleus size and drop of chromatin condensation in activated T cells. That was characteristic was confirmed by confocal imaging.

Conclusion. Raman spectroscopic imaging enables the recognition of activated T cells and represents a promising new approach in cell analysis.

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SIRTUINS PROMOTE BRAIN HOMEOSTASIS, PREVENTING ALZHEIMER'S DISEASE THROUGH TARGETING NEUROINFLAMMATION

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Both basic pathomechanisms underlying Alzheimer's disease and some premises for stipulating a possible preventive role of some sirtuins, especially SIRT1 and SIRT3, protective against Alzheimer's disease-related pathology, will be discussed during the presentation. Sirtuins can inhibit some processes that underlie Alzheimer's disease-related molecular pathology (e.g., neuroinflammation, neuroinflammation-related oxidative stress, Aβ aggregate deposition, and neurofibrillary tangle formation), thus preventing many of those pathologic alterations at relatively early stages of their development. Hence, the presentation provides details as to which mechanisms of sirtuin action may prevent the development of Alzheimer's disease, thus promoting brain homeostasis in the course of aging. In addition, the presentation provides a rationale for boosting sirtuin activity, both with allosteric activators and with NAD^+, in order to verify whether it can actually prevent Alzheimer's disease.

PROTECTIVE EFFECT OF RESVERATROL ON ASTROCYTE SIRT1 SECRETION (UPON NEUROINFLAMMATION) DEPENDS ON SYSTEMIC GLYCEMIA

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Neurovascular blood-brain barrier (BBB) precisely controls the flow of substances between the circulating blood and the central nervous system (CNS). Metabolic disorders, such as diabetes, and related hyperglycemia or (iatrogenic) hypoglycemia, can lead to disfunction and/or disruption of the BBB, and in consequence to neuroinflammation. This increases the risk for development of neurodegenerative disorders. Resveratrol (RSV) is one of the most studied natural anti-diabetic compounds, exhibiting anti-inflammatory and neuroprotective properties. It activates the sirtuin 1 gene (SIRT1) in human brain tissues, which results in inhibition of inflammatory processes, and of apoptosis in favor of autophagocytosis.

Aim. The objective of this study was to assess the protective effect of RSV on astrocyte SIRT1 secretion upon neuroinflammation in different glycemic backgrounds.

Materials and Methods. We established a two-component *in vitro* model of the BBB consisting of the microvascular compartment (MC) lined with endothelial cell line hCMEC/D3, and the brain compartment (BC) lined with human brain progenitor-derived astrocytes (ThermoFisher Scientific-Gibco; cat. no. N7805100). Neuroinflammation was induced by addition of 0,2 μM of lipopolysaccharide (LPS) to the BC of the BBB. RSV was administered at a concentration of 50 μM to the MC. The level of astrocyte-secreted SIRT1 was assessed using enzyme-linked immunosorbent assay (ELISA; Abcam, cat. no. ab171573).

Results. Neuroinflammation induced by LPS led to substantial decrease in SIRT1 secretion in all glycemic backgrounds. It was earliest observed in hyperglycemia. RSV partially counterbalanced the effect of LPS, and alleviated the decrease in SIRT1 secretion upon neuroinflammation. The neuroprotective effect of RSV was most remarkable in normoglycemic, and less pronounced in hypo- and hyperglycemic conditions.

Conclusions. To our knowledge this is the first systematic study on regulation of SIRT1 secretion by RSV-treated neuroinflammation in different glycemic backgrounds. Obtained results confirm that hypoglycemia per se is associated to increased SIRT1 secretion, comparing to normo- and hyperglycemia, and in short term could result in increased resistance to neuroinflammation, compared to hyperglycemia. In long run, however both abnormal (hypo- or hyper-)glycemic states are associated with higher sensitivity to neuroinflammation, and give worse prognosis of RSV-therapy effectiveness than normoglycemia.

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THE ROLE OF TNF-α AND ANTI-TNF-α AGENTS DURING PRECONCEPTION, PREGNANCY, AND BREASTFEEDING

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Tumor necrosis factor-alpha (TNF-α) is a multifunctional Th1 cytokine and one of the most important inflammatory cytokines. In pregnancy, TNF-α influences hormone synthesis, placental architecture, and embryonic development. It was also shown that increased levels of TNF-α are associated with pregnancy loss and preeclampsia. Increased TNF-α levels in complicated pregnancy draw attention to trophoblast biology, especially migratory activity, syncytialisation, and endocrine function. Additionally, elevated TNF-α levels may affect the maternal-fetal relationship by altering the secretory profile of placental immunomodulatory factors, which in turn affects maternal immune cells. There is growing evidence that metabolic/pro-inflammatory cytokines can program early placental functions and growth in the first trimester of pregnancy. Furthermore, early pregnancy placenta has a direct impact on fetal development and maternal immune system diseases that release inflammatory (e.g., TNF-α) and immunomodulatory factors, such as chronic inflammatory rheumatic, gastroenterological, or dermatological diseases, and may result in an abnormal release of cytokines and chemokines in syncytiotrophoblasts. Pregnancy poses a challenge in the treatment of chronic disease in patients who plan to have children. The activity of the disease, the impact of pregnancy on the course of the disease, and the safety of pharmacotherapy, including anti-rheumatic agents, in pregnancy should be considered.

COMPARATIVE ANALYSIS OF THE OCCURRENCE AND ROLE OF CX3CL1 FRACTALKINE) AND ITS RECEPTOR CX3CR1 IN HEMOPHILIC ARTHROPATHY AND OSTEOARTHRITIS

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Hemophilic arthropathy is characterized by recurrent bleeding episodes in patients with hemophilia leading to irreversible joint degeneration. The involvement of CX3CL1 (fractalkine, C-X3-C Motif Chemokine Ligand 1) and its receptor CX3CR1 (C-X3-C Motif Chemokine Receptor 1) was observed in the pathogenesis of numerous arthritis-associated diseases. Taking this into account we have performed a study investigating the role of the CX3CL1/CX3XR1 axis in hemophilic arthropathy.

Methods. The study was carried out using cases (n=20) of end-stage hemophilic arthropathy with a severe type of hemophilia A and control cases (n=20) diagnosed with osteoarthritis. The biofluids including blood serum and synovial fluid were obtained intraoperatively for the evaluation of CX3CL1 using ELISA test. Tissue specimens including articular cartilage and synovial membrane were similarly collected during surgery and stained immunohistologically using selected antibodies including anti-CX3CR1, anti-CD56, anti-CD68 and anti-CD31. Additionally, the analysis included the assessment of articular cartilage, synovial membrane and blood vessels morphology.

Results. In our study, we have documented increased average concentration of CX3CL1 in blood serum of study group (7.16 ± 0.53 ng/ml) compared to the control group (5.85 ± 0.70 ng/ml) without statistically significant difference in synovial fluid concentration at the same time. We have observed an increased macrophage presence with more marked proliferation and fibrosis of the synovial membrane in study group. Remaining results such as expression of CX3CR1 presence of NK (Natural killer) cells and larger surface area of blood vessels within the synovial membrane were noted also without statistical significance.

Conclusions. This study has demonstrated collective CX3CL1/CX3CR1 axis involvement in hemophilic arthropathy pathogenesis introducing new interesting diagnostics and therapeutic target.

THE ROLE OF KISSPEPTIN-10 IN THE REGULATION OF COLLAGEN METABOLISM IN THE MYOCARDIUM

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The aim of the present study was to determine the role of kisspeptin-10 (KiSS-10) in the regulation of collagen content in human cardiac fibroblasts *in vitro* and within the hearts of mice *in vivo.* In addition, an attempt was made to describe the mechanism of the effect of KiSS-10 on collagen metabolism.

The experiments were carried out on human cardiac fibroblast cell line and male BALB/c mice. *In vitro* model was used to determine intracellular collagen content, expression of α1 chains of procollagen type I and III (*Col1A1* and *Col3A1*), C-terminal propeptides of procollagen type I and III (PICP and PIIICP), metalloproteinases (MMP-1, -2, -9), tissue inhibitors of metalloproteinases (TIMPs 1-4), transforming growth factor β1 (TGF-β1) and phosphorylated focal adhesion kinase (FAK). *In vivo* studies were performed to assess the serum level of PICP and PIIICP as well as the collagen content and expression of *Col1A1* and *Col3A1* within the hearts of mice.

In vitro experiments with human cardiac fibroblasts and *in vivo* studies with animal model demonstrate that KiSS-10 significantly increases the content of collagen in the heart. KiSS-10 also enhances the level of phosphorylated FAK in human cardiac fibroblasts. However, inhibition of autophosphorylation of FAK abolishes the stimulatory effect of KiSS-10 on collagen deposition *in vitro*. These changes correlate with an increase in the level of the collagen turnover markers: PICP and PIIICP in human cardiac fibroblast culture medium *in vitro* as well as mouse PIIICP in serum *in vivo*. Moreover, kisspeptin-10 decreases the secretion of metalloproteinases (MMP-1, -2, -9) and elevates the release of their tissue inhibitors (TIMP-1, -2, -4) *in vitro*. KiSS-10 also increases the expression of *Col1A1* and *Col3A1* in human cardiac fibroblasts. However, exposure of fibroblasts to KiSS-10 does not affect release of TGF-β1 *in vitro*. KiSS-10 is involved in the regulation of collagen metabolism in the myocardium. Augmentation, by KiSS-10, of the collagen deposition is dependent on the protein synthesis elevation, inhibition of matrix metalloproteinases (increase of TIMPs release) or decrease of matrix metalloproteinases (MMP-1, -2, -9) concentration. The profibrotic activity of kisspeptin-10 is mediated by FAK activity and is not dependent on the release of TGF-β1. Thus, the results provide new insights into hormonal regulation of myocardial remodeling and point at KiSS-10 as the novel target for antifibrotic therapy.

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ACCUMULATION OF COLLAGEN IS REGULATED BY SUBSTRATE STIFFNESS VIA ACTIVATION OF α2β1 INTEGRIN, FAK AND SRC KINASE IN ATRIAL MYOFIBROBLASTS CULTURES

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Cardiac fibrosis is a major component of heart failure as well as development of arrhythmias. The aim of the study was to determine whether the substrate stiffness via stimulation of α2β1 integrin, focal adhesion kinase (FAK) and Src kinase is involved in regulation of collagen accumulation within the heart.

The study was performed on myofibroblasts derived from right atrium of patients with aortal stenosis. Cells were cultured on polyacrylamide gels with different stiffness. The cells, identified as myofibroblasts, expressed α-smooth muscle actin, vimentin and desmin. Procollagen Type I C-Terminal Propeptide (PICP, marker of collagen type I synthesis) was higher than in the cultures on stiff substrate. The expression of procollagen type I and III genes were no changed. Inhibition of α2β1 integrin by addition of TC-I 15 at concentrations of 10^{-7} mol/L and 10^{-8} mol/L increased collagen content in cells cultured on both substrates.

Similar results were obtained in cultures with silenced α2 integrin subunit by using siRNA. Administration of inhibitors of both FAK and Src kinase, also increased in collagen content of myofibroblasts culture. Tissue inhibitor of metalloproteinases 4 (TIMP-4) was elevated in soft gel cultures. Interleukin-6 (IL-6) level was augmented in soft gel cultures, but the level of Fibroblast Growth Factor 2 (FGF2) was not influenced.

Obtained results suggest that substrate stiffness via activation of α2β1 integrin FAK and Src kinase exerts regulatory effect on collagen accumulation in atrial myofibroblasts cultures.

FROM BIO-ACTIVITY PROFILING OF GUANIDINES TO THE DISCOVERY OF POTENT MUSCARINIC M_2R/M_4R ANTAGONISTS

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Aim. Based on the previously obtained data of the guanidine series the lead H_3R antagonist - ADS1017 [1], was selected for further structural optimization. The idea was to replace the flexible seven methylene linker with a semi-rigid 1,4-cyclohexylene or *p*-phenylene substituted group. These simple structural modifications of the H_3R antagonist led to the emergence of additional pharmacological effects, some of which unexpectedly decreased the electrically-evoked contractility of ileum smooth muscles.

Material and Methods. All newly-synthesized compounds were evaluated as antagonists/agonists at histamine H_3R on guinea pig ileum (gpH_3R) and muscarinic M_2R/M_3R (gpM_2R/M_3R). Then, a radioligand displacement assay in membrane fractions of HEK-293 cells stably expressing human H_3R (hH_3R) was performed. Finally, hM_1-hM_5 radioligand binding experiments were carried out for selected ligands.

Results. During the *ex vivo* assay on the guinea pig ileum, decreasing the electrically-evoked contractility of ileum smooth muscles was noticed. This effect could be related to H_3R agonism as well as to the action the muscarinic receptors present in the tested tissue. The tests carried out on the guinea pig ileum showed that the rigidity of the seven-carbon alkyl chain, not only decreases affinity at the histamine H_3R, but above all significantly increases affinity at muscarinic M_2R and M_4R.

Conclusion. This work presents the discovery of novel potent muscarinic receptor antagonists identified during the search for more active H_3R ligands [2]. Synthesized guanidine derivatives are a novel chemotype of muscarinic receptor antagonists, binding to the hM_2R and hM_4R in nanomolar concentration ranges.

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NEW MOLECULES AS OPIOID LIGANDS

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Introduction of Oliceridine by FDA into the market in 2021 opened new chapter in search for new opioid ligands. Use and overuse of opioids in the western world lead to geometrical growth of dependence for such group of narcotics. New informations concerning specific and biased agonizm could be crucial for developing new “pain killers” with less or not side effects.

Search for new scaffold could be based on the structure of PZM-21 containing urea moiety. Functional testing revealed selective activation of the G protein pathway without β-arrestin pathway turning on. That biased agonism could be one way of activity of new opioids. Another could be allosteric interaction with MOP (OP3, mu opioid receptor), positive or negative, decreasing active doses of typical opioids. Example of the negative allosteric modulator for MOP receptor is sodium cation, which interrupt water channel crucial for stabilization of the active for of receptor. Obtained by us 1-(imidazolin-2-yl)-urea derivatives exhibited negative allosteric model of action but in the same time they were also strongly increasing action of morphine in under threshold doses.

Results will be presented and discussed in a view of possible mechanism.

ON THE ENHANCEMENT OF ANTIDEPRESSANT-LIKE EFFICACY OF HALLUCINOGENIC DRUGS BY MGLU2/3 RECEPTOR ANTAGONIST OR NEGATIVE ALLOSTERIC MODULATORS (NAMS)

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Hallucinogenic substances from various groups (ketamine, scopolamine, psilocybin) exert antidepressant effects both in the preclinical and clinical studies. The group II metabotropic (mGlu) receptor antagonists also show antidepressant efficacy in animal studies.
Here we demonstrate that the joined administration of sub-effective doses of ketamine and mGlu2/3 receptor antagonist LY341495 produced a pronounced and long lasting antidepressant effects.
A sub-effective dose of scopolamine HBr (0.03 mg/kg) together with LY341495 induced a clear antidepressant-like effect in the forced swim test (FST). Moreover a selective M1 muscarinic receptor antagonist VU0255035 produced a dose dependent antidepressant effects which were potentiated by a low dose of mGlu2 receptor NAM VU60001966
Psilocybin (0,5 mg/kg) + LY341495 (0,3 mg/kg) (doses which are not active by itself) induces a prolonged antidepressant-like effects in mice in tail suspension test (TST) 24, 72 h or 7 days after single dose administration. In the novelty suppressed feeding test (NSFT) the effect was observed 24 h after a single administration of both drugs.
The results shows that the hallucinogenic drugs from various groups exert antidepressant-like activity which is enhanced by mGlu2/3 receptor antagonists/NAMs .

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THE IMPACT OF E-98, NOVEL H3R ANTAGONIST, ON THE PROCESS OF NOCICEPTION AND MORPHINE EFFECTIVENESS IN A NERVE INJURY-INDUCED NEUROPATHIC PAIN MODEL

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Neuropathic pain is a growing public health problem across the world. A variety of available pharmacological medications (such as opioids) do not provide adequate pain relief. Therefore, there is still a strong need for new analgesics. Growing body of evidences have shown that histaminergic system might be a key modulator of neuropathic pain, especially interesting target is histamine H_3R receptor. The expression of H_3R within the nervous system was observed on neurons, as well as glial cells, which are strongly related with neuropathic pain formation.

The aim of this study was to determine, whether novel H_3R antagonist, E-98, would affect symptoms of neuropathic pain and modulate morphine-induced analgesia. Moreover, the goal of our research was to determine if changes in microglial cells activation are engaged in analgesic action of H_3R antagonist during neuropathy.

Methods. Albino Swiss mice were subjected to chronic constriction injury (CCI) of the sciatic nerve (model of neuropathic pain). The effects of systemic E-98 (1, 5, 10, 20 mg/kg) administration on mechanical (von Frey) and thermal (cold plate, tail flick) stimuli in naive and CCI-exposed (14 days after injury) animals were evaluated. Moreover, we examined the effect of combination treatment with E-98 (5 mg/kg) and opioid receptor agonist (morphine; 5 mg/kg) on pain symptoms. Moreover, behavioural observations were correlated with changes in microglial cells activation (Iba1, TMEM119, CD208 markers) in the lumbar spinal cord (Western blot).

Results. Here, we revealed the analgesic potency of E-98 in naive (tail flick test) and CCI-exposed (von Frey, cold plate and tail flick tests) animals after single i.p. injection, which was dose- and time-dependent. Moreover, after chronic E-98-treatment (10 mg/kg; twice daily, for 7 days) we observed strong analgesic effects of the new antagonist. Interestingly, when administered together at non-effective doses, E-98 and morphine, strongly reduced symptoms of neuropathic pain. Our biochemical data revealed decreased level of microglial activation at the spinal cord level after E-98 chronic treatment.

Conclusion. Our work provides the evidences for the analgesic potency of novel H_3R antagonist and its beneficial properties for morphine effectiveness. Based on our data we suggest that analgesic effects of E-98 partially appear due to the modulation of microglial cells activation within the CNS. Our studies help us to better understand the molecular underpinnings of the neuropathic pain and will improve chronic pain therapy.

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REPURPOSING OF POTENTIAL ANTIPSYCHOTICS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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The aim of the study was to evaluate a suitability of a multi-target antipsychotic, D2AAK1 and its derivatives for the treatment of neurodegenerative diseases.

Materials and Methods. We used rational structure-based design methods to develop multi-target ligands of aminergic G protein-coupled receptors which can be used to treat mental diseases, such as schizophrenia, depression or anxiety. Detailed investigation of in vitro and in vivo profiles of the selected compounds indicated that they may be repurposed for the treatment of neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease.

Results. It was determined that the selected compounds displayed an increase in proliferation of mouse hippocampal neuron cells [1]. In the case of neuroblastoma cells, there is no increase in cell proliferation. Moreover, the cells incubated with the compounds are more elongated, characterized by longer dendrites as compared to the control. Next, the compounds lower the levels of reactive oxygen and nitrogen species (ROS and NOS, respectively) and in the test using the Ca^{2+} probe cause the decrease of calcium level with the increasing cell incubation time. The compounds do not cause the influx of calcium ions into the cell, which also protects the cell against the excitotoxicity process [2,3]. In the behavioral tests the selected compounds exhibit pro-cognitive properties in the passive avoidance test and novel object recognition test in mice both after acute and chronic administration. Finally, the PASS software was applied to search for possible additional molecular targets to explain the observed in vitro activity of the compounds and the identified targets are under experimental validation.

Conclusions. The preliminary results are promising and indicate that the studied compounds may be successfully repurposed for the treatment of neurodegenerative diseases.

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THE SEARCH FOR CHOLINESTERASE INHIBITORS AMONG HISTAMINE H_3 RECEPTOR LIGANDS

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Background. The histamine H_3 receptor (H_3R) is widely distributed in the brain, especially in the parts connected with memory and cognition. Its blockade increases the release of histamine itself as well as other neurotransmitters (e.g., acetylcholine). In the past years, in addition to selective H_3R antagonists/inverse agonists multi-target (mostly dual acting) ligands were also obtained, exhibiting antagonism/inverse agonism at the H_3R while simultaneously affecting other biological targets such as inhibition of cholinesterases (ChEs) [1].

Aim. The search for multi-target H_3R ligands among a series of acetyl- and propionyl phenoxyalkyl (homo)piperidine derivatives.

Materials and Methods. Ether derivatives of (un)substituted (homo)piperidines were synthesized and tested *in vitro*. H_3R affinity was assessed in a radioligand binding assay using [^{3}H]N^{α}-methylhistamine as radioligand in HEK293 cells stably expressing human H_3R ($h$$H_3R$). ChEs inhibitory activity was estimated spectrophotometrically by the Ellman method using acetylcholinesterase (AChE) from *electric eel* and butyrylcholinesterase (BuChE) from *horse serum* [2].

Results. All tested compounds exhibited good affinities for $h$$H_3R$ (K_i values from 13 to 123 nM). The inhibitory activities for ChEs were variable and depended mainly on the substituent in the benzene ring (acetyl or propionyl) and the length of the alkyl linker (5 or 6 carbon atoms). Acetyl derivatives mostly showed no inhibitory activity at concentrations of 10 micromoles above 70% against both ChEs. In contrast, propionyl derivatives inhibited (IC_{50} in low micromolar range) mainly BuChE.

Conclusions. Among propionyl derivatives of homopiperidine were found the most potent ChEs inhibitors. 1-(4-((5-Azepan-1-yl)pentyl)oxy)phenyl)propan-1-one was the most BuChE inhibitor with IC_{50} of 0.55 μM, whereas 1-(4-((6-(azepan-1-yl)hexyl)oxy)phenyl)propan-1-one proved to be the most potent AChE inhibitor (IC_{50} = 1.06 μM).

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FUNCTIONAL SELECTIVITY OF NEW HISTAMINE H_4 RECEPTOR LIGANDS FROM THE GROUP OF ALKYL-AND CYCLOALKYL-1,3,5-TRIAZINE DERIVATIVES

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Aim. Functional selectivity affects two main areas in the drug discovery: ligand detection and drug optimisation. From the point of view of drug screening it is not sufficient to rely only on one functional assay [1]. Histamine H_4 receptor (H_4R) is $G_{i/o}$ protein-coupled receptor, that can transmit signalling through G protein dependent pathway or by activation of β-arrestin. In the peripheral blood cells H_4R activation leads to the intercellular calcium^{2+} efflux in the $G_{i/o}$ - and $G_{q/11}$-dependent manner [2]. It is known, that H_4R ligands may present functional selectivity (i.e., JNJ7777120 exhibits partial agonist properties in reference to β-arrestin signalling pathway of H_4R [3]). Therefore, the group of H_4R ligands (1,3,5-triazine derivatives) were tested for their functional selectivities, by focusing on three intercellular second messengers: cAMP, β-arrestin and Ca^{2+}.

Material and Methods. Compounds were synthesized according to the previously described methods [4]. Binding at H_4R was tested using [^{3}H]histamine displacement assay with membrane preparation of S*f*9 cells expressing human H_4R, co-expressed with G protein $G_{αi2}$ and $G_{β1γ2}$ subunits. For determination of β-arrestin activity of H_4R ligands the LiveBLAzer™, cell based assay and Tango-H4-bla U2OS cells were used (both from ThermoFisher Scientific). The level of cAMP was determined using LANCE Ultra cAMP Detection Kit and CHO-K1 cells stably expressing human H_4R. Measurement of Ca^{2+} level was performed using histamine H_4 AequoZen cell line and AequoScreen Starter Kit (both from PerkinElmer).

Results. Most compounds showed high affinities for human H_4R with K_i values < 400 nM. The selected structures tested in the β-arrestin recruitment assay, the cAMP accumulation assay and the Ca^{2+} efflux assay showed antagonist activities in all tested pathways. Compound TR-DL-13 was the most active in the binding (K_i = 63 nM), and proved to be also the most potent antagonist in the β-arrestin recruitment (IC_{50} = 10 nM) and Ca^{2+} efflux assay (IC_{50} = 0.016 nM).

Conclusions. Structure-activity relationships (SAR) observed in the affinity test was mostly confirmed in the functional studies (i.e, the most affine ligand TR-DL-13 proved to be the most active antagonist). However few differences can be pointed between H_4R binding and β-arrestin recruitment in the group of straight and branched alkyl derivatives. The knowledge of these differences inspire to further and deeper investigation in this group of compounds.

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DIFFERENCES BETWEEN PROTON PUMP INHIBITORS AND HISTAMINE H2 RECEPTOR ANTAGONISTS - ARE THEY REALLY IMPORTANT?

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Proton pump inhibitors (PPIs) and histamine 2-receptor antagonists (H_2RAs) are two classes of drugs that are used to treat conditions associated with excess gastric acid production, including peptic ulcer disease, dyspepsia, gastroesophageal reflux disease (GERD), gastroduodenal injury associated with non steroidal anti-inflammatory drugs (NSAIDs) and Zollinger-Ellison syndrome.

Their mechanisms of action are different. PPIs work by blocking gastric H^+K^+ATPase, which is responsible for pumping H^+ ions from within gastric parietal cells into the gastric lumen, where they react with Cl^- ions to form hydrochloric acid. H_2RAs work by inhibiting the interaction of histamine, a potent stimulus of gastric secretion, with the parietal cell histamine H_2 receptor, thus reducing gastric acid production.

The results of blocking proton pump action are more potent and long lasting, so intragastric pH is maintained above 4 for many hours. The action of H_2RAs is faster, but shorter lived and tolerance develops after longer use. This property allows them to be used in “on-demand” management of GERD, while H_2RAs are not recommended for gastroprotection during treatment with NSAIDs.

PPIs, but not H_2RAs, after long term administration can reduce absorption of calcium, magnesium and ferrum. Both drugs influence vitamin B_{12} absorption when administered as long-term therapy.

The strong acid suppression by PPIs can enhance infectious complications, not only intestinal infections including *Clostridioides difficile* and small intestinal bacterial overgrowth – SIBO, but also pneumonia, especially in Intensive Care Units. H_2RAs are preferred alternative in this situation in mechanically ventilated patients. Neither PPIs nor H_2RAs change the course and do not improve nor deteriorate the outcomes of COVID-19 patients.

LACTATE OPERATES AS THE HCA1 LIGAND WITH HDAC INHIBITORY ACTIVITY TO MODULATE CELLULAR NHEJ COMPONENTS AND RESTRICT TRANSDUCTION RATE OF RETROVIRAL VECTORS

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Vaginal and ectocervical microbiota of the female genital tract protects against pathogen colonization through competition for adherence, production of antimicrobial substances and secretion of high amounts of lactate. Recently, we have reported the lactate-driven mechanisms by which lactate under physiological conditions modulates the activity of DNA repair machinery, particularly NHEJ system and its key protein DNA-PKcs in the cervical epithelial cells. These pathways include stimulation of surface-specific lactate hydroxycarboxylic acid receptor 1 (HCA1/GPR81) and decrease of nuclear chromatin compactness by inhibition of HDAC (histone deacetylase). Interestingly, DNA-PKcs is indispensable for proper retroviral DNA integration in cell host genome.

Aim. The objective of the present study was to assess the possible role of lactate in retroviral DNA integration via modulation of DNA-PKcs cellular activity/localisation.

Results. Our study was the first to demonstrate that stimulation of cervical cancer cells with lactate suppresses the transduction rate of lentiviruses. We have shown that incubation of cervical epithelial cells with 20 mM of L- or D-lactate decreased the transduction rate of engineered retroviral vectors along with inversely proportional DNA-PKcs nuclear increase. Stimulation of cells with either HCA1 agonist 3,5-DHBA or HDAC inhibitor sodium butyrate mimicked in part the effects of L-lactate.

Conclusion. Our study suggests that L- and D-lactate present in the uterine cervix may play a role in the mitigation of viral integration in cervical epithelium and thus, restrict the viral oncogenic and/or cytopathic potential.

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