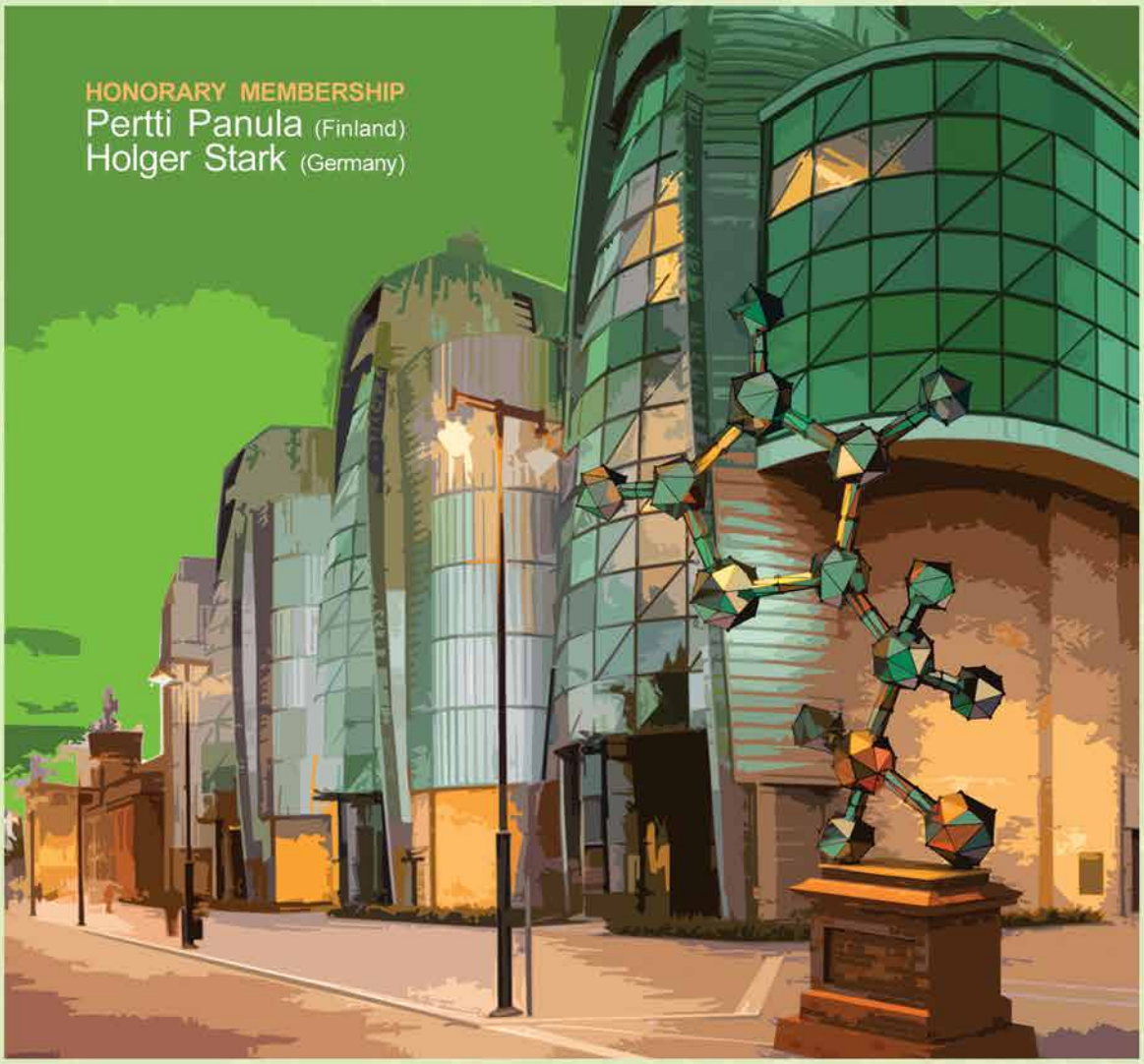
Biogenic Amines and Related Biologically Active Compounds

Aminy biogenne i pokrewne związki o wysokiej aktywności biologicznej



25-27 October 2018
Lodz, Poland

XVIIth Conference of the Polish Histamine Research Society
XVII Konferencja Polskiego Towarzystwa Badań Nad Histaminą

Invited Speakers:

Enzo Agostinelli (Italy), Nicholas Carruthers (USA), Philippe De Deurwaerdère (France), Madeleine Ennis (UK), Hiroyuki Fukui (Japan), Jerzy Jochem (Poland), Barbara Malinowska (Poland), Beatrice Passani (Italy), Pertti Panula (Finland), Holger Stark (Germany)

Dear Attendees,

Welcome to our 17th Conference devoted to Biogenic Amines and Related Biologically Active Compounds. This year's conference is dedicated to the memory of late Professor Kenji Tasaka (who died 07. 10. 2018) for his outstanding contribution to histamine research.

Professor Tasaka's connection to histaminologists in Poland was long-standing and friendly. The many honours he obtained worldwide included the Copernicus Medal given by the Polish Academy of Science and an honorary membership of our Society. It is always sad to pay our last respects to our teachers and mentors. We have done this previously in relation to other honorary members of the Polish Histamine Research Society, namely Professors: Richard W. Schayer (09.11. 1997), Czeslaw Maslinski (11.07.2002) and Walter Schunack (09.04.2011).

However, on a more positive note, I am happy to announce that the General Assembly today approved the appointment of two new honorary members of the Polish Histamine Research Society: Professor Pertti Panula, University of Helsinki, Finland, and Professor Holger Stark, Heinrich Heine University of Düsseldorf, both are not only well known, leading scientists promoting knowledge on histamine but also good friends to Polish histaminologists, often attending our conferences and sharing their findings and their experience with us.

I hope you all will enjoy the time spent gathering and discussing new information on recent advances in research on histamine, dopamine, serotonin and related other biologically active compounds whilst listening to 11 lectures and 28 shorter presentations which cover important aspects of biogenic amine functions.

Finally, please also enjoy the social events and most importantly - make friends here and come back in two years' time!

W. Agnieszka Fogel

President
Polish Histamine Research Society

Scientific Programme

Thursday, October 25th 2018

14:00 Arrival, accommodation
Ambasador Centrum Hotel, Piłsudskiego 29 St., 90-307 Lodz

15:30 General Assembly of members of the Polish Histamine Research Society,
Conference room "Maltańska"

17:00 – 19:00 Registration
Ambasador Centrum Hotel, Reception Hall

17:00 – 19:00 Poster mounting,
Conference room "Maltańska"

19:00 **Welcome Addresses**
Conference room "Maltańska"

Prof. Dr. W. Agnieszka Fogel,
President of the Polish Histamine Research Society

Prof. Dr. Jurek Olszewski,
Dean of the Faculty of Military Medicine, the Medical University of Lodz

HONORARY MEMBERSHIP CEREMONY
Perrti Panula (Helsinki, Finland), laudation by W. Agnieszka Fogel
Holger Stark (Düsseldorf, Germany), laudation by Katarzyna Kieć-Kononowicz

L1 **THE HISTORY OF HISTAMINE RESEARCH IN THE EHRS**
Lecture by Madeleine Ennis,
The Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK

20:00 – 22:00 Welcome Reception
Ambasador Centrum Hotel, Restaurant

Friday, October 26th 2018

8:30 – 10:30 **Session I**
Chair: Jerzy Jochem, Anna Stasiak

8:30 – 9:00
L2 **PLASTICITY AND INTERACTIONS OF THE HISTAMINERGIC AND HYPOCRETIN SYSTEMS**
Pertti Panula, Svetlana Semenova, Maria Sundvik, Henri Puttonen, Diego Baronio, Yu-Chia Chen
Department of Anatomy and Neuroscience Center, University of Helsinki, Helsinki, Finlandv

9:00 – 9:30
L3 **TARGETING PROTEIN-PROTEIN INTERACTIONS OF AMPA CHANNELS**
Nicholas I. Carruthers
Janssen Research & Development, L.L.C., San Diego, USA

9:30 – 10:00
L4 **DISTINCT BRAIN MAPPING OF DOPAMINERGIC AND SEROTONERGIC METABOLISMS AT REST IN IMPULSIVITY, RISK-TAKING AND UNFLEXIBLE BEHAVIOUR IN RATS**
Philippe De Deurwaerdère[1], Marion Rivalan, Aurélie Fitoussi, Françoise Dellu-Hagedorn[1]
[1]Université de Bordeaux, Bordeaux, France; CNRS, Institut des Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

10:00 – 10:20
O1 **EXPRESSION OF DOPAMINE AND SEROTONIN RECEPTORS IN BLOOD MONONUCLEAR CELLS AS A BIOMARKER FOR SCHIZOPHRENIA**
Adam Wysokiński[1], Ewa Szczepocka[1], Anna Łucka[1], Katarzyna Sobierajska[2], Justyna Agier[3], Elżbieta Kozłowska[3], Ewa Brzezińska-Błaszczyk[3]
[1]Department of Old Age Psychiatry and Psychotic Disorders, Medical University of Lodz, Lodz, Poland; [2]Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland; [3]Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

10:20 – 10:25
P1 **MULTI-FUNCTIONAL LIGAND D2AAK4 AS A POTENTIAL ANTIPSYCHOTIC**
Agnieszka A. Kaczor[1,2], Andrea G. Silva[3], Katarzyna M. Targowska-Duda[4], Grażyna Biała[5], Marian Castro[3]
[1]Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Lab, [4]Department of Biopharmacy and [5]Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy with Division for Medical Analytics, Lublin, Poland; [2]School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [3]Department of Pharmacology, Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Santiago de Compostela, Spain

10:25 – 10:30
P2 **NEW AGONIST OF 5-HT$_7$ RECEPTOR SIGNIFICANTLY DIMINISHES NEUROPATHIC PAIN SYMPOMS AND POTENTIATES MORPHINE ANALGESIA IN THE MOUSE**
Agata Ciechanowska[1], Katarzyna Popiolek-Barczyk[1], Adam S. Hogendorf[2], Agata Hogendorf[2], Andrzej J. Bojarski[2], Joanna Mika[1]
[1]Department of Pain Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland; [2]Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

10:30 – 10:50 Coffee /Tea
Discussions by Posters

10:50 – 12:45 **Session II**
Chair: Dariusz Matosiuk, Dorota Łażewska

10:50 – 11:20
L5 **BINDING KINETICS AT HISTAMINE AND DOPAMINE RECEPTOR SUBTYPES**
David Reiner[1], Annika Frank[1], Dóra J Kiss[2], György M Keserű[2], Holger Stark[1]
[1]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Duesseldorf, Germany; [2]Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

11:20 – 11:40
O2 **STEP FORWARD IN SEARCH FOR NOVEL HISTAMINE H$_3$ RECEPTOR LIGANDS: 4-PYRIDYL-PIPERAZINE DERIVATIVES WITH PROMISING MULTIDIRECTIONAL PHARMACOLOGICAL ACTIVITY**
Katarzyna Szczepańska[1], Tadeusz Karcz[1], Szczepan Mogilski[2], Kamil J. Kuder[1], Stefanie Hagenow[3], Magdalena Kotańska[2], Holger Stark[3], Bassem Sadek[4], Katarzyna Kieć-Kononowicz[1]
[1]Department of Technology and Biotechnology of Drugs, [2]Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland; [3]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; [4]Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirate

11:40 – 12:00
O3

HISTAMINE H_3 RECEPTOR LIGANDS WITH MONOAMINE OXIDASES INHIBITORY ACTIVITY

Kamil J. Kuder[1], Dorota Łażewska[1], Agnieszka Olejarz-Maciej[1], Maria Kaleta[1], Marek Bajda[2], Agata Siwek[3], Tadeusz Karcz[1], Agata Doroz-Płonka[1], Urszula Cichoń[1], Katarzyna Kieć-Kononowicz[1]

[1]Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland; [2]Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland; [3]Department of Pharmacobiology, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland

12:00 – 12:20
O4

EFFECTS OF PHARMACOLOGICAL MODULATION OF THE HISTAMINE H_3 RECEPTOR ON THE PROCESS OF NOCICEPTION IN NEUROPATHIC PAIN

Katarzyna Popiolek-Barczyk[1,2], Dorota Łażewska[1], Gniewomir Latacz[1], Agnieszka Olejarz[1], Holger Stark[3], Wioletta Makuch[2], Katarzyna Kieć-Kononowicz[1], Joanna Mika[2]

[1]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Krakow, Poland; [2]Institute of Pharmacology, Polish Academy of Sciences, Department of Pain Pharmacology, Krakow, Poland; [3]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

12:20 – 12:50
L6

IN SILICO APPROACHES TO BIASED SIGNALLING AND ALLOSTERIC MODULATION OF OPIOID RECEPTORS

Damian Bartuzi, Agnieszka A. Kaczor, Dariusz Matosiuk

Department of Synthesis and Chemical Technology of Medicinal Substances with Computer Modelling Lab, Medical University of Lublin, Lublin, Poland

12:50 – 12:55
P3

MONOAMINE OXIDASE B INHIBITION OF NOVEL ANALOGS AND DERIVATIVES OF 1-[3-(4-*TERT*-BUTYL-PHENOXY)PROPYL]PIPERIDINE

Agnieszka Olejarz[1], Urszula Cichoń[1], Dorota Łażewska[1], Tadeusz Karcz[1], Agata Siwek[2], Holger Stark[3], Katarzyna Kieć-Kononowicz[1]

[1]Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland; [2]Department of Pharmacobiology, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland; [3]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Duesseldorf, Germany

13:00 – 14:15 Lunch

14:15 – 15:55 **Session III**

Chair: Urszula Mazurek, Agnieszka A. Kaczor

14:15 – 14:45
L7

COMBINATION THERAPY WITH SUPPESSORS OF TWO PATHOLOGICAL MECHANISMS IN POLLINOSIS

Hiroyuki Fukui[1], Hiroyuki Mizuguchi[2], Yoshiaki Kitamura[3], Noriaki Takeda[3]

Departments of [1]Molecular Studies for Incurable Diseases and [3]Otorhinolaryngology, Tokushima University Graduate School of Biomedical Sciences; [2]Department of Pharmacology, Faculty of Pharmacy, Osaka Ohtani Univertsity

14:45 – 15:05
O5

ASSESSMENT OF SELECTED PARAMETERS OF IMMUNE RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES IN THE PRESENCE OF GRAPHENE

Ilona Dudek, Marta Skoda, Dariusz Szukiewicz

Department of General and Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

15:05 – 15:25
O6

SERUM CONCENTRATIONS OF OPG AND RANKL IN RHEUMATOID ARTHRITIS IN DIFFERENT BIOLOGIC THERAPIES

Katarzyna Romanowska-Próchnicka[1,4], Marzena Olesińska[1], Agnieszka Paradowska-Gorycka[2], Małgorzata Mańczak[3], Anna Felis-Giemza[1], Dariusz Szukiewicz[4], Sławomir Maśliński[4]

[1]Department and Polyclinic of Systemic Connective Tissue Diseases, Institute of Rheumatology, Warsaw, Poland; [2]Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland; [3]Department of Epidemiology and Health Promotion, Institute of Rheumatology Warsaw, Poland; [4]Department of General and Experimental Pathology, CEPT laboratory, Medical University of Warsaw, Poland

15:25 – 15:30
P4

THE OPPOSITE EFFECTS OF LEPTIN AND ADIPONECTIN ON MAST CELLS INFLAMMATORY RESPONSE

Justyna Agier, Elżbieta Kozłowska, Ewa Brzezińska-Błaszczyk, Paulina Żelechowska

Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

15:30 – 15:35
P5

THE EFFECT OF FUNGAL ANTIGENS ON MAST CELL ACTIVITY

Urszula Godzik, Paulina Żelechowska, Elżbieta Kozłowska,
Ewa Brzezińska-Błaszczyk, Justyna Agier

Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

15:35 – 15:40
P6

EXPRESSION OF THE FUNCTIONAL LEPTIN RECEPTOR ON THE MAST CELLS

Paulina Żelechowska[1], Magdalena Wiktorska[2], Sylwia Różalska[3], Olga Stasikowska-Kanicka[4], Małgorzata Wągrowska-Danilewicz[4], Justyna Agier[1], Ewa Brzezińska-Błaszczyk[1]

[1]Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland; [2]Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland; [3]Department of Industrial Microbiology and Biotechnology, University of Lodz, Lodz, Poland; [4]Department of Nephropathology, Medical University of Lodz, Lodz, Poland

15:40 – 15:45
P7

SHORT-TERM HYPOXIA REGULATES LAD2 MAST CELL ADHESION TO FIBRONECTIN THROUGH INTEGRIN α5β1 ACTIVATION

Joanna Pastwińska[1,2], Aurelia Walczak-Drzewiecka[1], Jarosław Dastych[1]

[1]Laboratory of Cellular Immunology, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland; [2]Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

15:45 – 15:50
P8

EXPRESSION OF GENES ASSOCIATED WITH HISTAMINE RECEPTORS IN PATIENTS WITH PSORIATIC ARTHRITIS TREATED WITH ANTI-TNF BIOLOGICAL THERAPY

Agata Kaźmierczak[1], Dominika Wcisło-Dziadecka[2], Celina Kruszniewska-Rajs[1], Magdalena Kimsa-Dudek[3], Barbara Strzałka-Mrozik[1], Urszula Mazurek[1]

[1]Department of Molecular Biology, [2]Department of Skin Structural Studies, [3]Department of Nutrigenomics and Bromatology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland

15:50 – 15:55
P9

THE RELATIONSHIP BETWEEN HISTAMINERGIC SYSTEM AND IL12/23 SIGNALING PATHWAY IN NHDF CELL CULTURE AFTER ADALIMUMAB TREATMENT

Beniamin Grabarek[1], Dominika Wcisło-Dziadecka[2], Joanna Gola[1], Barbara Strzałka-Mrozik[1], Celina Kruszniewska-Rajs[1], Urszula Mazurek[1]

[1]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland; [2]Department of Skin Structural Studies, Chair of Cosmetology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland

15:55 – 16:50 **Session IV**

Chair: Katarzyna Kieć-Kononowicz, Barbara Malinowska

15:55 – 16:25
L8

ENZYMATIC SPERMINE METABOLITES CAUSE APOPTOSIS IN CANCER CELLS DETECTED BY FLOW CYTOMETRY, REAL TIME RT-PCR AND PROTEOMIC ANALYSES
Yuta Kanamori, Enzo Agostinelli
Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza University of Rome, Rome, Italy

16:25 – 16:30
P10

EVALUATION OF CHANGES IN THE EXPRESSION PROFILE OF GENES INVOLVED IN MITOPHAGY IN COLORECTAL CANCER
Martyna Bednarczyk[1], Celina Kruszniewska-Rajs[2], Nikola Zmarzły[2], Urszula Mazurek[2], Małgorzata Muc-Wierzgoń[1]
[1]Department of Internal Medicine, School of Public Health in Bytom, Medical University of Silesia in Katowice, Poland; [2]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland

16:30 – 16:35
P11

DIVERSITY OF EXPRESSION OF GENES ENCODING CASPASES DEPENDING ON THE CLINICAL STAGE OF COLORECTAL CANCER
Klaudia Simka[1], Bartłomiej Skowronek[1], Małgorzata Muc-Wierzgoń[1], Teresa Kokot[1], Urszula Mazurek[2]
[1]Department of Internal Medicine, School of Public Health in Bytom, Medical University of Silesia, Katowice, Poland; [2]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland

16:35 – 16:40
P12

MELATONIN AND CIRCADIAN RHYTHM REGULATION IN ENDOMETRIAL CANCER
Ewelina Hermyt[1], Nikola Zmarzły[2], Agnieszka Jęda-Golonka[1], Katarzyna Szczepanek[1], Celina Kruszniewska-Rajs[2], Joanna Gola[2], Urszula Mazurek[2], Andrzej Witek[1]
[1]Department of Gynecology and Obstetrics, School of Medicine in Katowice; [2]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland

16:40 – 16:45
P13

MEMBRANE AND NUCLEAR MELATONIN RECEPTORS IN ENDOMETRIAL CANCER
Agnieszka Jęda-Golonka[1], Ewelina Hermyt[1], Nikola Zmarzły[2], Katarzyna Szczepanek[1], Celina Kruszniewska-Rajs[2], Joanna Gola[2], Andrzej Witek[1], Urszula Mazurek[2]
[1]Department of Gynecology and Obstetrics, School of Medicine in Katowice; [2]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland

16:45 – 16:50
P14

POTENTIAL CROSSTALK BETWEEN MELATONIN AND EPITHELIAL MESENCHYMAL TRANSITION IN ENDOMETRIAL CANCER
Nikola Zmarzły[1], Ewelina Hermyt[2], Agnieszka Jęda-Golonka[2], Katarzyna Szczepanek[2], Celina Kruszniewska-Rajs[1], Joanna Gola[1], Andrzej Witek[2], Urszula Mazurek[1]
[1]Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec; [2]Department of Gynecology and Obstetrics, School of Medicine in Katowice, Medical University of Silesia in Katowice, Poland

16:50 Sandwich /Coffee /Tea
Discussions by Posters

18:00 Bus transfer to the Musical Theatre in Lodz

18:30 Musical "Les Misérables", the Musical Theatre in Lodz

Transfer back

ca 22:00 Dinner
Ambasador Centrum Hotel, Restaurant

Saturday, October 27th 2018

9:15 – 10:55 **Session V**
Chair: W. Agnieszka Fogel, Krzysztof Walczyński

9:15 – 9:45
L9

HISTAMINE AND ENDOCANNABINOIDS, TWO PHYLOGENETICALLY OLD SYSTEMS, TALK TO EACH OTHER IN THE BRAIN AND PERIPHERAL ORGANS
M. Beatrice Passani
Department of Health Sciences - University of Florence, Viale Pieraccini 6, Firenze (I)

9:45 – 10:15
L10

ROLE OF THE ENDOCANNABINOID SYSTEM IN HYPERTENSION
Barbara Malinowska
Department of Experimental Physiology And Pathophysiology, Medical University of Białystok, Poland

10:15 – 10:45
L11

EFFECTS OF HISTAMINE H_3 RECEPTORS BLOCKAGE IN HAEMORRHAGIC SHOCK IN RATS
Jerzy Jochem
Department of Physiology, School of Medicine with the Division of Dentistry, Medical University of Silesia, Katowice, Poland

10:45 – 10:50
P15

ASSESSMENT OF PROGRESS IN TREATMENT OF CHRONIC PRESSURE ULCERS USING METALLOPROTEINASE AND CYTOKINE GENES EXPRESSION
Anna Polak[1,4], Grażyna Janikowska[2], Barbara Strzałka-Mrozik[3], Urszula Mazurek[3], Małgorzata Paczuła[1,5], Ewa Hordyńska[5], Tomasz Ickowicz[1,5], Cezary Kucio[1]
[1]Department of Physiotherapy in Internal Organs Diseases, The Jerzy Kukuczka Academy of Physical Education in Katowice; [2]Department of Analytical Chemistry, [3]Department of Molecular Biology, Medical University of Silesia in Katowice; [4]Medical and Rehabilitation Center "Technomex" in Gliwice; [5]Uppersilesian Rehabilitation Center "Repty" in Tarnowskie Góry, Poland

10:50 – 10:55
P16

RESPONSE OF SELECTED CELLULAR PARAMETERS OF THE IMMUMOLOGICAL SYSTEM IN THE COURSE OF PROTON PUMP INHIBITORS THERAPY
Katarzyna Kosikowska-Skowron, Jakub Wronecki, Halina Cichoż-Lach, Barbara Skrzydło-Radomańska
Department of Gastroenterology, Medical University, Lublin, Poland

10:55 – 11:30 Coffee /Tea
Discussions by Posters

11:30 – 12:45 **Session VI**
Chair: Dariusz Szukiewicz, Joanna Gola

11:30 – 11:50
O7

GRAPHENE INTERACTIONS WITH HUMAN ENDOTHELIUM
Marta Skoda, Ilona Dudek, Dariusz Szukiewicz
Department of General and Experimental Pathology, CEPT Laboratory, Medical University of Warsaw, Warsaw, Poland

11:50 – 11:55
P17

CHANGES IN SEROTONIN AND TNF-INDUCED PATHWAYS IN ADSC CO-CULTURED WITH LESC CELLS
Joanna Gola, Bartosz Sikora, Celina Kruszniewska-Rajs, Aleksandra Skubis-Sikora, Barbara Strzałka-Mrozik, Urszula Mazurek
Department of Molecular Biology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland

11:55 – 12:00
P18

EXPRESSION OF MELATONIN RELATED GENES IN MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE AFTER CO-CULTURE WITH LIMBAL EPITHELIAL STEM CELLS

Aleksandra Skubis-Sikora, Bartosz Sikora, Celina Kruszniewska-Rajs, Joanna Gola, Urszula Mazurek
Department of Molecular Biology, School of Pharmacy with Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland

12:00 – 12:05
P19

GENES RELATED TO HISTAMINE IN THE PROCESS OF ADSC DIFFERENTIATION AFTER CO-CULTURE WITH LESC

Bartosz Sikora, Aleksandra Skubis-Sikora, Joanna Gola, Celina Kruszniewska-Rajs, Urszula Mazurek
Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

12:05 – 12:25
O8

ACTIVITY BASED ANOREXIA IN FEMALE WISTAR RATS – A PILOT STUDY

Magdalena Kurnik-Łucka[1], Paulina Stach[1], Piotr Podlasz[2], Kamil Skowron[1], Krzysztof Wąsowicz[2], Agnieszka Baranowska[1], Veronika Aleksandrovych[1], Krzysztof Gil[1]
[1]Department of Pathophysiology, Jagiellonian University Medical College, Krakow, Poland; [2]Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

12:25 – 12:45
O9

BENEFITS AND HEALTH RISKS OF FOOD SUPPLEMENTS CONSUMPTION

Przemysław Rzodkiewicz[1,2], Leszek Markuszewski[3]
[1]Department of Functional Foods, Chief Sanitary Inspectorate, Warsaw, Poland; [2]Department of General and Experimental Pathology, Medical University of Warsaw, Poland; [3]University of Social Sciences, Lodz, Poland

12:45 Closing Ceremony of the XVII-th Conference of the Polish Histamine Research Society

13:00 Lunch

THE HISTORY OF HISTAMINE RESEARCH IN THE EHRS

Madeleine Ennis

The Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, 97 Lisburn Rd, Belfast BT9 7BL, UK; e-mail: m.ennis@qub.ac.uk

Histamine is an old mediator – first synthesized in 1907 by Windaus and Vogt and in 1910 two groups (Barger and Dale, Ackermann) found it in nature. In the following decades its role in anaphylaxis was revealed. In the 1930s, the first antihistamines (H_1 receptor antagonists) were synthesized, and in 1942 Antergant (phenbenzamine, RP 2339) became the first agent to be used in man. In 1953, came the seminal discovery that histamine was stored in mast cells (Riley and West).

In 1971, a Satellite Symposium on histamine was held in Lodz (Poland) after the XXV International Congress of Physiological Sciences in Munich (Germany). At this meeting it was decided that the European Branch of the Histamine Club should meet annually starting with the inaugural meeting in Paris in 1972. There have now been 47 meetings in 17 different countries of the renamed European Histamine Research Society (EHRS).

The first meeting started at an extremely exciting time for histamine research! Although the existence of a second histamine receptor had been proposed by Ash and Schild in 1966, the first H_2 receptor antagonists were described in 1972 (Black and co-workers).

Now we know that there are 4 histamine receptors and the EHRS meetings have witnessed enthralling talks and poster presentations on the role of histamine in many areas, far exceeding its role in the allergic response. The society is characterised by excellent collaborative research between scientists from different countries. This talk will illustrate some of the exciting and novel findings presented at our meetings as our knowledge of histamine's functions and its receptors increased.

PLASTICITY AND INTERACTIONS OF THE HISTAMINERGIC AND HYPOCRETIN SYSTEMS

Pertti Panula, Svetlana Semenova, Maria Sundvik, Henri Puttonen, Diego Baronio, Yu-Chia Chen

Department of Anatomy and Neuroscience Center, University of Helsinki, Helsinki, Finland

The histaminergic system comprises neurons in the posterior hypothalamus and nerve fibers extending to most areas of the brain. The hypocretin system consists of neurons in hypothalamic perifornical area and connections with most ascending activating systems. These two systems are essential to maintain normal wakefulness, and the hypocretin neurons are among the most powerful activators of the histamine neurons. Hypocretin neurons degenerate in narcolepsy in humans, whereas the number of histaminergic neurons has been reported to increase significantly. Both histamine and hypocretin neuron systems are well conserved in zebrafish.

The purpose of this study was to investigate the mechanisms of interactions between the histaminergic and hypocretin systems using genetic, behavioral, biochemical, and microscopic methods.

Normal and gene-modified zebrafish were used in this study. Fish lacking the presenilin 1 (psen 1) gene were produced using the TILLING method, fish lacking histamine H3 receptor (hrh3) were produced in the laboratory using the CRISPR/Cas method. Quantitative behavioral methods, HPLC, confocal microscopy and qPCR were used.

Zebrafish lacking psen1 showed during the larval stage deficient dark-induced flash response, which is also characteristic of histamine-deficient fish. At adult stages they showed characteristic increased thigmotaxis (swimming along the edges of the arena), which is typical of high brain histamine activity. Counting of histamine neurons showed that at larval stages the number of histamine neurons was abnormally low, whereas the number in adult fish was about 50% higher than in control fish. The system thus showed abnormal plasticity. This was found to be due to abnormally low expression of notch1 transcription factor, the processing of which is dependent on psen1. Inhibition of translation of histidine decarboxylase (hdc) decreased the number of hypocretin neurons in larval zebrafish brain, whereas overexpression of hdc increased in a dose-dependent manner the number of hypocretin neurons. To reveal the receptor responsible for histaminergic control of hypocretin neuron numbers, hrh3 KO fish were produced. In these fish, the number of hypocretin neurons was significantly lower than in control fish. Thus, histamine seems to regulate the developing hypocretin neurons at least through hrh3.

In zebrafish, the histaminergic neuron system shows significant bidirectional life-long plasticity of neuron numbers, which is regulated at least in part by the gamma-secretase system component psen1, and notch1, which is processed by gamma-secretase. Since notch1 is an important regulator of stem cells, it is possible that the new neurons in adult fish brain originate from the stem cell pool rather than existing mature neurons through phenotype switching. Hypocretin neuron numbers in zebrafish are partly regulated by histamine through hrh3.

TARGETING PROTEIN-PROTEIN INTERACTIONS OF AMPA CHANNELS

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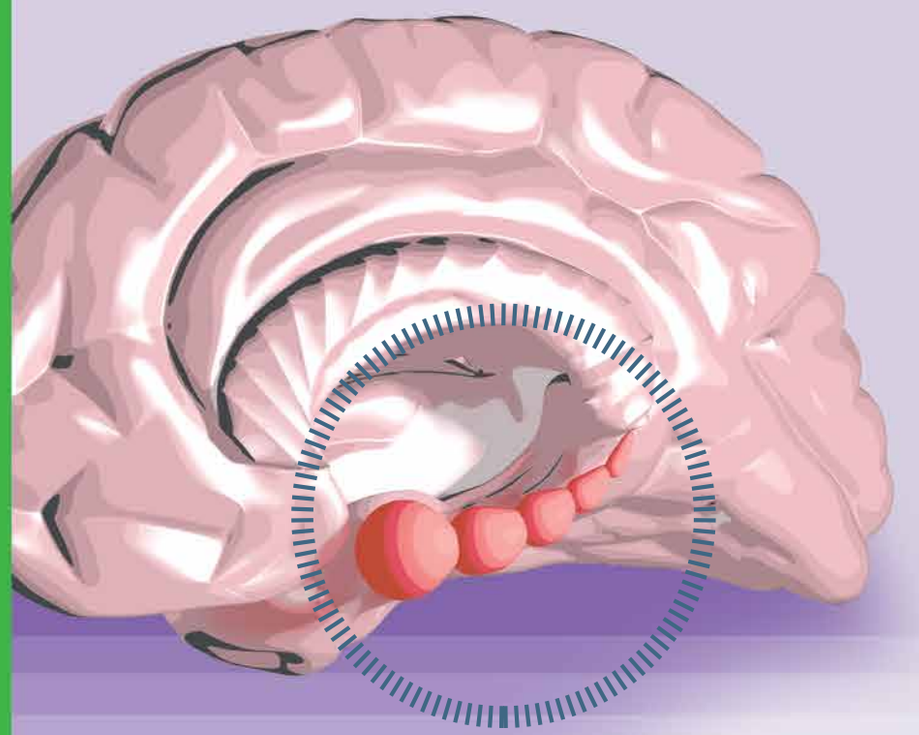
Glutamate mediates the majority of fast synaptic transmission in the central nervous system (CNS) via activation of ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Although AMPA receptors (AMPARs) are widely expressed throughout the CNS, their activity can be modulated by numerous auxiliary proteins including transmembrane regulatory proteins (TARPs), which are often localized in distinct brain regions. In particular, TARP-γ8 is expressed primarily in the hippocampus, a key component of the limbic system. By selectively inhibiting neurotransmission within this brain region, negative modulators of TARP-γ8 dependent AMPARs exhibit strong anticonvulsant effects in rodent seizure models without the motor impairment associated with nonselective AMPAR antagonists. The preclinical pharmacology for JNJ-55511118 an AMPA receptor negative modulator with exquisite selectivity for TARP-γ8 together with a second-generation series of 5-pyridyloxindoles will be presented.



DISTINCT BRAIN MAPPING OF DOPAMINERGIC AND SEROTONERGIC METABOLISMS AT REST IN IMPULSIVITY, RISK-TAKING AND UNFLEXIBLE BEHAVIOUR IN RATS

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Several impulse control disorders such as ADHD, mania, personality disorders or substance abuse share common behavioural traits, like impulsiveness, risk taking or inflexible behaviour. These disorders are treated with drugs targeting dopamine (DA) and/or serotonin (5-HT). However, the patient's monoamine imbalance that these neurotransmitters compensate is unclear. This study aims to investigate the patterns of DA and 5-HT metabolisms at rest within selected brain regions related to inter-individual variability in six main components of impulsivity/compulsivity (anticipatory hyperactivity, premature responses, delay discounting, risk taking, perseverations, flexibility).

35 naive rats were used in this study. They were screened for their behavioural responses in five distinct tests addressing the six component of impulsivity/compulsivity: the Fixed Consecutive Number of 16 lever press schedule (FCN16) (premature responses), the multiple Fixed-Interval/Extinction schedules of reinforcement (FI-EXT) (anticipatory hyperactivity and perseveration), the Delay Discounting Task (DDT) (impulsive choice), the Rat Gambling Task (RGT) (behavioural flexibility), the light-dark emergence test (risk taking). According to their performance in each behaviour they were divided into sub-groups by extracting subgroups of individuals with low or high scores according to the upper and the lower terciles in each task (n =12 ± 2), the remainder constituting an intermediate group (n=12 ± 2). After the completion of the behavioural experiment, the content of monoamines and metabolites was evaluated using high pressure liquid chromatography coupled to electrochemical detection in 20 brain areas (10 cortical and 10 subcortical areas). Metabolisms corresponded to the ratios DOPAC/DA for DA and 5-HIAA/5-HT for 5-HT and the results were expressed for both ratios as the mean ± SEM in each subgroup (low versus high behavioural scores).

Distinct patterns of 5-HT and DA metabolisms were revealed according to the behavioural traits. Except for hyperactive responses, lower control of actions was mainly associated with a lower DA or 5-HT metabolism in prefrontal and/or subcortical areas (i.e. in orbitofrontal cortex (DA), amygdala and anterior cingulate cortex (5-HT) for inflexible and risk prone rats). A further analysis using correlative analysis revealed complete distinct monoaminergic mapping between well adapted and maladaptive responders in each test.

Our results reveal the complex nature of behavioural traits related to impulse control disorders through their associated monoaminergic networks at rest. These results pave the way for understanding the link between mental disorders and drug therapeutic actions.

Invited Lecture

BINDING KINETICS AT HISTAMINE AND DOPAMINE RECEPTOR SUBTYPES

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Growing evidence recommends to incorporate the concept of drug-target residence times within drug development and screening programs. For many targets, systematic research for binding kinetics have been sparingly performed and reported, as in case of the histamine H_3 receptor [1], that may be caused by relatively laborious methods using radiolabeled ligands. Alternatively, fluorescent methods based on Foerster resonance energy transfer have been reported recently, but application of fluorescence polarization to drug-target kinetics is not described. Thus, we established a radiolabel-free, real-time resolving method that is compatible to high-throughput-screening programs with the objective to explore the underlying binding kinetics.

In addition to the binding kinetic at histamine H_3 receptors, the dissociation behaviors at dopamine D_3 receptors have been investigated for aripiprazole and cariprazine with kinetic experiments using a radiolabeled [^{3}H]spiperone [2]. At the D_3 receptor, aripiprazole exhibits a slow monophasic dissociation, while cariprazine displays a rapid biphasic behaviour.

With these kinetic findings novel agents may be developed that display improved dissociation behaviors from the aminergic receptors to further investigate this effect on in vivo profiles.

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IN SILICO APPROACHES TO BIASED SIGNALLING AND ALLOSTERIC MODULATION OF OPIOID RECEPTORS

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In human, G protein-coupled receptors (GPCRs) constitute the largest family of receptors and one of the largest families of proteins in general. They are responsible for a vast part of signal transmission within organism, as well as for sensing external stimuli such as light or odour. Their function was once thought to be plain and straightforward, being considered as simple one-function relays, recognizing particular stimulus and responding to it with one particular intracellular signal. Today it is known, however, that nature designed GPCR structures for complex signal processing, with one single receptor molecule capable of inducing different signalling cascades in response to different ligands – particular ligands can 'bias' signalling toward particular effector. Understanding this complexity is crucial for design of modern drugs selective toward selected signalling pathway rather than receptor only, which is believed to bring less side effects. GPCRs can also be allosterically modulated by small molecules, which is also a promising strategy for design of safer drugs. However, engineering such molecules is problematic for many reasons, including probe dependence (modulators affect different transmitters in a very different way) and species selectivity (drug candidate performing well in rodent models may behave differently in humans).

Opioid receptors are among the most intensively investigated GPCRs in allosteric and/or biased drug design. Such drugs could greatly improve current therapies, cursed with dangerous side effects. In recent years, some prototypical allosteric and biased compounds were reported, e.g. BMS986122, Ignavine, TRV-130, PZM21 or SHR9352. Also, atypical ligands with no protonable nitrogen atom and unknown mechanism of action were reported. To understand their mechanisms and allow for rational design of further analogues, we decided to apply computational methods. We created a native-like in silico environment, including μ opioid receptor in complex with G protein, immersed in a raft-like asymmetric membrane. The system was then set in motion with molecular dynamics simulations. Number of simulations with different allosteric modulators, G protein-biased or β-arrestin-biased agonists and/or atypical ligands bound to the receptor were performed. Importantly, our simulations were performed on the human receptor model in a native-like environment, so the results may eventually help to overcome difficulties related to differences in properties of drug candidates in mouse models and humans.

COMBINATION THERAPY WITH SUPPESSORS OF TWO PATHOLOGICAL MECHANISMS IN POLLINOSIS

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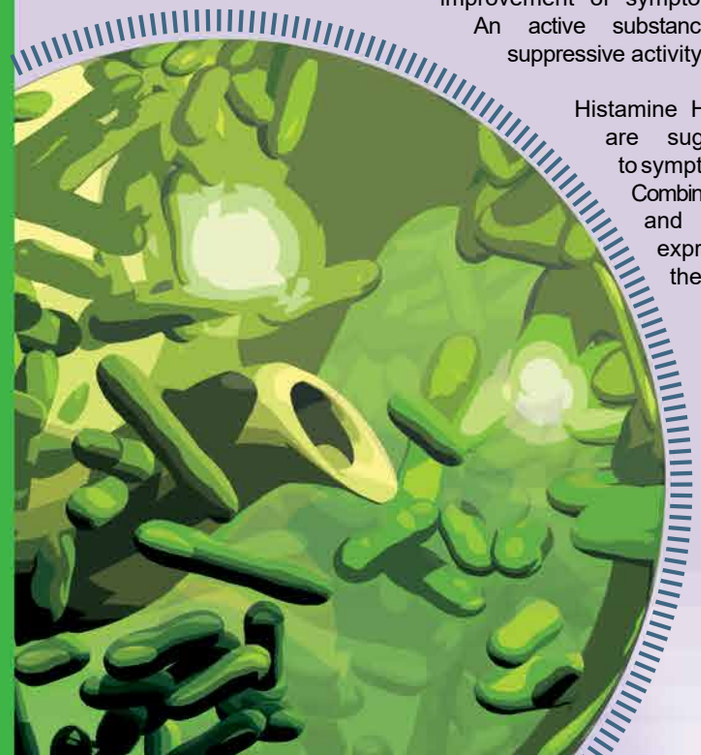
Antihistamines are insufficient for high-level alleviation of pollinosis symptoms. It was attempted to elucidate the mechanisms of pollinosis symptoms and also the development of novel therapeutic strategy for allergic diseases.

Real-time PCR was applied in *in vitro* and *in vivo* studies. HeLa cells and RBL 2H3 cells were used for the in-vitro studies at the molecular level. Toluene 2, 4-diisocyanete (TDI)-sensitized Brown Norway nasal hyper-sensitivity model rats were used for in vivo studies.

Stimulation of histamine H_1 receptor (H_1R) induced the up-regulation of H_1R gene expression. Correlation between symptoms and H_1R mRNA level in nasal mucosa was observed in patients with pollinosis. Although elevated H_1R mRNA level was normalized by antihistamine treatment in TDI-sensitized model rats, symptoms were only partially reversed. Combination treatment of an antihistamine and Awa-tea showed high-level improvement of symptoms in allergic model rats. An active substance from Awa-tea showed suppressive activity on IL-9 gene expression.



Histamine H_1 receptor and IL-9 genes are suggested to be sensitive to symptoms of nasal hypersensitivity. Combination therapy with antihistamines and suppressor of IL-9 gene expression is promising as novel therapeutics for pollinosis.

ENZYMATIC SPERMINE METABOLITES CAUSE APOPTOSIS IN CANCER CELLS DETECTED BY FLOW CYTOMETRY, REAL TIME RT-PCR AND PROTEOMIC ANALYSES

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Neuroblastoma (NB) is the most common cancer in infancy and most frequent cause of death from extracranial solid tumors in children. Therefore, besides the current treatments that include chemotherapy, surgery, and radiation, new therapies need to be developed. The in situ formation of cytotoxic polyamine metabolites by bovine serum amino oxidase (BSAO) is a recent approach in cancer therapy. It was demonstrated that BSAO and spermine (SPM) addition to cancer cells induces cell growth inhibition by apoptosis caused by H_2O_2 and aldehydes, produced by the oxidative reaction [1].

The induction of apoptosis in NB cells was evaluated by flow cytometry after Annexin V-FITC labelling and DNA staining with propidium iodide, by real time RT-PCR, transmission electron microscopy (TEM), proteomic and bioinformatics studies.

The percentages of Annexin V-positive cells matched quite well with that of cells showing hypodiploid sub-G1 peak. An increase in mitochondrial membrane depolarization (MMD) was found in neuroblastoma cells treated with the enzymatic system. We analysed by real time RT-PCR the transcript of some genes involved in the apoptotic process, to determine possible down or up regulation of mRNAs after the treatment of the SJ-N-KP cell line with BSAO and SPM. The experiments were carried out considering the pro-apoptotic genes P53, PUMA and CASPASE-3. After treatment with BSAO and SPM, the SJ-N-KP cells displayed increased mRNA levels for all these pro-apoptotic genes. Interestingly, the pro-apoptotic Sirt-1 inhibitor microRNA miR-34a significantly increases in SJ-N-KP cells treated with BSAO and SPM. These data support the concept that BSAO/SPM treatment induces high levels of apoptosis. Previously we demonstrated that multidrug resistant (MDR) colon adenocarcinoma cells (LoVo DX) are more sensitive than the corresponding wild type cells (LoVo WT) to H_2O_2 and aldehydes [2]. Transmission electron microscopy observations showed evident mitochondria alterations. The mitochondrial activity was checked by flow cytometry studies, labelling cells with the probe JC1. After treatment with BSAO/SPM the cells showed a marked increase in MMD. In order to have more information on the effect of BSAO/SPM in cancer cells, a proteomics approach was performed using LoVo cells and prostate cancer (LNCaP) cells treated with and without BSAO/SPM. In total 721 unique proteins were identified in LNCaP cells (more than 4700 in LoVo cells) of which 40 were differentially expressed by more than 1.3 folds. The canonical pathways that exhibited the largest differences between BSAO treated and untreated cells in the presence of SPM include mitochondrial dysfunction.

We conclude that the mechanism of the cytotoxicity of BSAO/SPM is partly related to mitochondrial dysfunction.

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HISTAMINE AND ENDOCANNABINOIDS, TWO PHYLOGENETICALLY OLD SYSTEMS, TALK TO EACH OTHER IN THE BRAIN AND PERIPHERAL ORGANS

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The central nervous system and viscera constitute a functional ensemble, the gut-brain axis that allows bidirectional information flow that contributes to the control of feeding behaviour, appetite, memory, and the response to stress and pain. Recent research on the gut-brain axis has revealed the contributions of extensive neuronal networks and chemical factors, amongst which is the anandamide monounsatured analogue, oleoylethanolamide (OEA) an endocannabinoid synthesised in the intestine upon ingestion of dietary fat. OEA reduces food intake through a mechanism that involves recruitment of peripheral sensory afferents and activation of central pathways that utilize histamine as a neurotransmitter [1]. Also, OEA improves memory retention in several tasks, presumably to optimize food searching and the ability to remember the context associated with food availability. We showed that in this case as well, histamine neurotransmission is necessary for OEA to unfold its procognitive effects [2, 3]. These results suggest that brain histamine serves as a relay station integrating peripheral signals and central functions to influence the emotional value of different experiences and to control energy expenditure and eating behaviour.

We recently found that the histamine and OEA, two phylogenetically ancient molecules, interact with each other in other organs as well to control homeostatic functions. OEA levels in the small intestine of various vertebrate species, including fish, snakes and rodents, rise after feeding and activate peroxysome proliferator activated receptor-α (PPAR-α) [4]. Feeding regulates OEA production in other organs as well. In the liver, OEA levels rise in the fasting state and fall after feeding [5], in a fashion opposite to the intestine and adipose tissue. The physiological function of hepatic OEA is presumably the stimulation of fatty-acid oxidation and the enhancement of ketone body production (ketogenesis) in live rats [6]. Ketogenesis is an adaptive metabolic response to prolonged nutrient insufficiency, which takes place primarily in liver mitochondria. We recently found that mast cells contribute to the local biosynthesis of OEA via a paracrine mechanism that involves secretion of histamine into the hepatic portal circulation and stimulation of liver H_1 receptors [7]. Genetic or pharmacological manipulations that disrupt this process do not affect lipolysis, but markedly attenuate fasting-induced ketogenesis. These results reveal an unexpected new function for mast cell-derived histamine in the regulation of fasting-induced liver ketogenesis, a key node of bioenergetics homeostasis. We raise the intriguing possibility that dysfunctions in this paracrine signaling mechanism might contribute to metabolic disorders.

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ROLE OF THE ENDOCANNABINOID SYSTEM IN HYPERTENSION

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Phytocannabinoids, i.e. preparations of *Cannabis sativa* have been used for centuries not only for recreational purposes but also for effective treatment of hypertension or glaucoma. The endocannabinoid system comprises cannabinoid CB_1 and CB_2 receptors, their endogenous ligands [e.g. anandamide (AEA)] and enzymes that synthesize and degrade these ligands [e.g. AEA is degraded mainly by fatty acid amide hydrolase (FAAH)]. All components of the endocannabinergic system were determined in the human cardiovascular system. The endocannabinoid system is overactivated in arterial, pulmonary and portal hypertension [1, 2]. It is suggested that its therapeutic modulation may be beneficial in arterial hypertension. However, many of the studies reported so far have concentrated on the acute injection of AEA, FAAH inhibitors or other cannabinoid ligands and their hypotensive response that was much stronger in anaesthetized hypertensive rats than in their normotensive controls. It has not been unambiguously confirmed by chronic studies. Moreover, the precise mechanism(s) of hypotensive action of cannabinoid-triggered compounds are very complex and still controversial. Various levels of endocannabinoids or their effects have been determined in primary (spontaneously hypertensive rats) and in various models of secondary hypertension. The cannabinoid-dependent changes in hypertension were connected with alternations in cannabinoid signalling in the central nervous system (nucleus tractus solitarius, rostral ventrolateral medulla, paraventricular nucleus of the hypothalamus), heart, vessels, their interaction with angiotensin II or with the modification of arterial baroreflex or oxidative stress. Importantly, in hypertensive patients, the higher level of anandamide and genetic polymorphisms association with arterial hypertension have been demonstrated. However, these data need to be confirmed in larger groups. Thus, the therapeutic potential of drugs modulating the endocannabinoid system in hypertension needs additional preclinical and clinical studies and conformations.

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Invited Lecture

EFFECTS OF HISTAMINE H_3 RECEPTORS BLOCKAGE IN HAEMORRHAGIC SHOCK IN RATS

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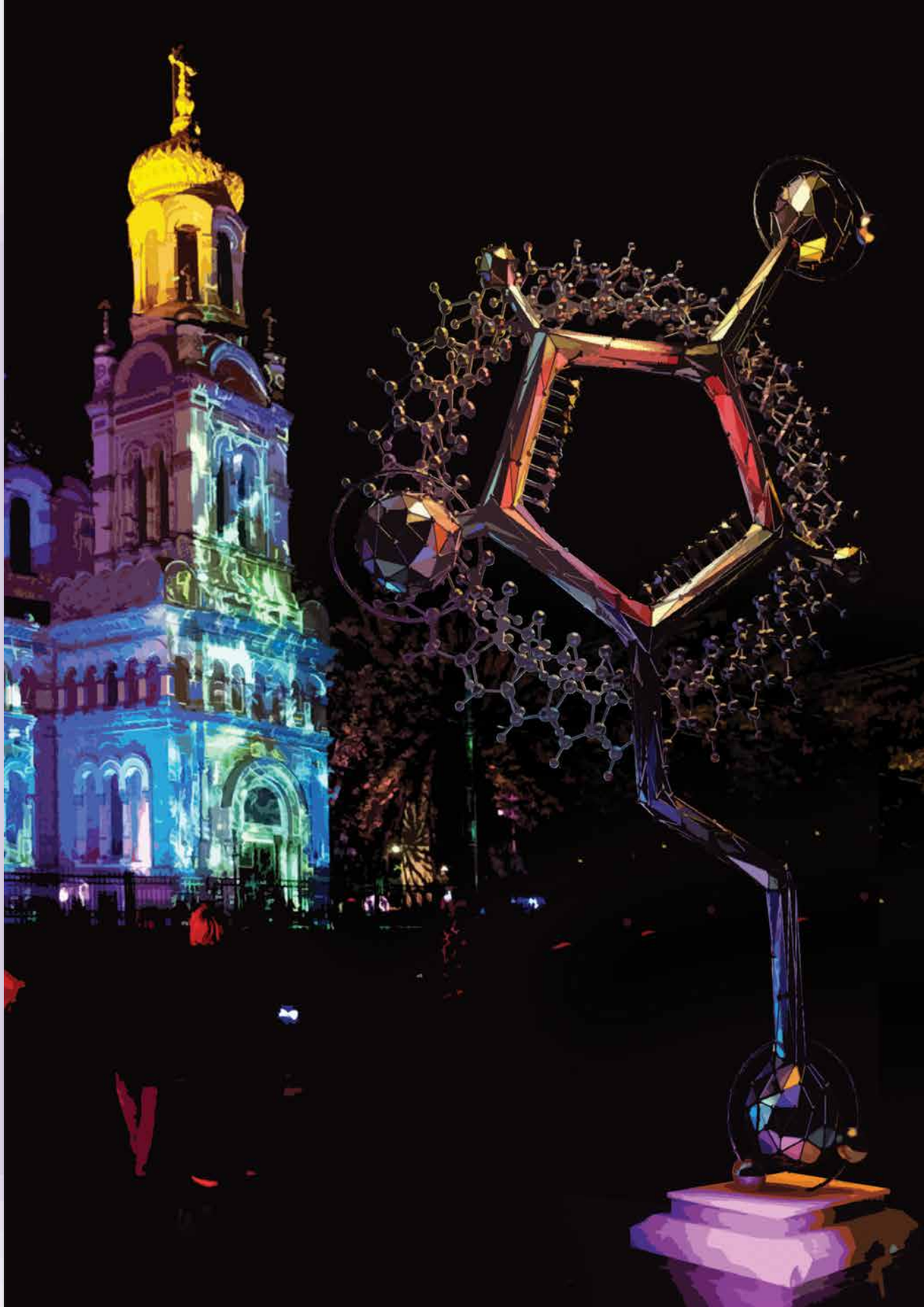
Haemodynamic response to acute blood loss can be divided into two phases – (1) a reflex-induced increase in the sympathetic system activity (sympathoexcitatory phase) (2) a decrease in the sympathetic activity (sympathoinhibitory phase). Prolonged tissue hypoperfusion, especially in the second phase of regulation, and subsequent reperfusion induce immunological changes which may lead to multiorgan dysfunction/systemic inflammatory response syndrome in haemorrhagic shock.

Histamine H_3 receptors are present on postganglionic endings of the sympathetic neurons and are able to regulate the synthesis and release of postganglionic neurotransmitters. As demonstrate studies in vitro, activation of H_3 receptors located on postganglionic endings innervating vessels leads to a vasodilatation. In addition, the neurogenic vasopressor tone can be modulated via H_3 receptors.

Our studies demonstrated that a blockage of H_3 receptors at the sympathoinhibitory phase of regulation induces a long-lasting increases in blood pressure and peripheral blood flows, with an increase in survival. The effects were inhibited after peripheral chemical sympathectomy with 6-hydroxydopamine or intravenous pre-treatment with α_1-adrenoceptor antagonist prazosin, but not with β-adrenoceptor blocker propranolol. The pressor effect was accompanied by reduced increases in plasma proinflammatory cytokines, such as TNF-alpha, IL-1alpha, IL-1beta, IL-12 and IFN-alpha.

In conclusion, (1) peripheral H_3 receptors are able to influence cardiovascular system function in haemorrhagic shock and (2) blockage of histamine H_3 receptors – probably due to the improvement in tissue perfusion – decreases hypoxia/reperfusion-induced immunological changes.



EXPRESSION OF DOPAMINE AND SEROTONIN RECEPTORS IN BLOOD MONONUCLEAR CELLS AS A BIOMARKER FOR SCHIZOPHRENIA

Adam Wysokiński[1], Ewa Szczepocka[1], Anna Łucka[1], Katarzyna Sobierajska[2], Justyna Agier[3], Elżbieta Kozłowska[3], Ewa Brzezińska-Błaszczyk[3]

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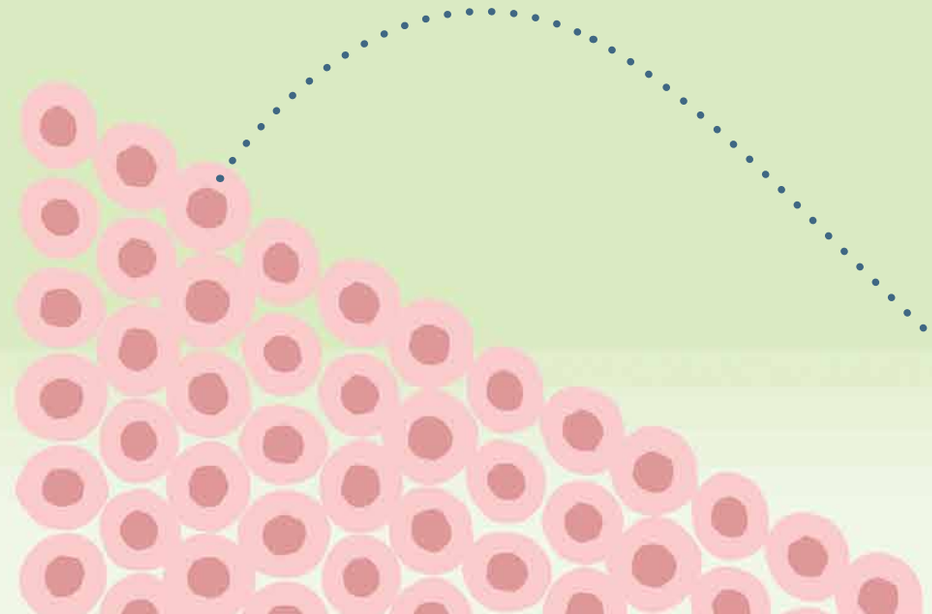
The aim of this research was to determine the mRNA expression profile of dopamine receptors D1, D2, D3 and D4 and serotonin receptors 5HT1A, 5HT2A and 5HT3A in peripheral blood mononuclear cells in schizophrenia. Most of these receptors seem to be involved in the pathophysiology of schizophrenia and could be used as a potential diagnostic and treatment-predictive biomarker. We have recruited twenty-seven patients with schizophrenia and 29 healthy controls. The expression of dopamine and serotonin receptors was measured using quantitative RT-PCR in peripheral blood mononuclear cells. Clinical symptoms were assessed using PANSS and CDSS scales.

All study subjects underwent anthropometric and body composition measurements. The major finding is that the schizophrenia group demonstrated significantly higher mRNA expression of D2 and D4 receptors ($p < 0.001$), and significantly lower mRNA expression of D3, 5HT1A, 5HT2A and 5HT3A receptors ($p < 0.01$). The two groups also presented different profile of ratios of mRNA expression of these receptors.

In conclusion, schizophrenia patients display a distinct pattern in the expression of dopamine and serotonin receptors, at mRNA levels. Hence, rather than individual receptors, a specific set of these receptors may constitute a potential biomarker for this condition.



MULTI-FUNCTIONAL LIGAND D2AAK4 AS A POTENTIAL ANTIPSYCHOTIC

Agnieszka A. Kaczor[1,2], Andrea G. Silva[3], Katarzyna M. Targowska-Duda[4], Grażyna Biała[5], Marian Castro[3]

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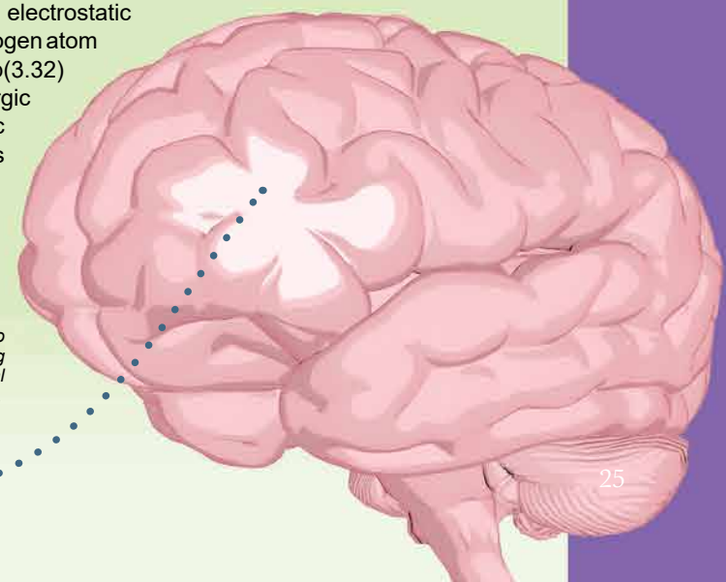
The modern approach to drug design and discovery for the treatment of complex diseases, like neurodegenerative diseases, cancer and many psychiatric disorders, involves searching for medicinal substances which fulfil criteria of several pharmacophores, instead of acting on a single molecular target. Indeed, in complex psychiatric illnesses, including schizophrenia, selective single-target drugs have been to a great extent a failure. The pharmacological profile of clozapine reflects the molecular pathogenesis of schizophrenia, which involves cross-talk of many neurotransmitter systems (especially dopaminergic, serotonergic, adrenergic and glutamatergic). The new paradigm in drug design and discovery is to search for compounds which modulate the activity of several molecular targets simultaneously. To achieve this, it is necessary to identify structural features that link important classes of drug targets, which will enable the design of drugs with the desired selectivity profiles.

We identified a novel multi-target ligand of aminergic GPCRs, D2AAK4, using structure-based virtual screening [1]. D2AAK4 possesses nanomolar or low micromolar affinity to D_1, D_2, D_3, 5-HT_{2A} and 5-HT_7 receptors, making it an ideal candidate for a multi-target drug. Here we present homology modeling, molecular docking and molecular dynamics of D2AAK4 and its molecular targets and animal studies of D2AAK4 as a potential antipsychotic. The main contact of D2AAK4 and all the receptors studied is the electrostatic interaction between the protonatable nitrogen atom of the ligand and the conserved Asp(3.32) as typical for orthosteric ligands of aminergic GPCRs. We demonstrated antipsychotic and, importantly, procognitive properties of D2AAK4 in mouse models.



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NEW AGONIST OF 5-HT7 RECEPTOR SIGNIFICANTLY DIMINISHES NEUROPATHIC PAIN SYMPTOMS AND POTENTIATES MORPHINE ANALGESIA IN THE MOUSE

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Neuropathic pain is a chronic state, lowering life quality. This type of pain is difficult to treat and present therapies are insufficient. 5-HT_7 receptor (the member of the serotonin receptor family) is a promising target for development new drugs to provide an effective pain therapy. 5-HT_7 is widely expressed in the central nervous system, also in the regions related to nociceptive transmission. The aim of our study was to investigate the analgesic effect of newly synthesized potent compound, which is a full agonist of 5-HT_7 receptor (AGH-194) in mouse model of neuropathy (chronic constriction injury - CCI to the sciatic nerve). We assessed influence of AGH-194 on nociception and morphine analgesia nerve in mice 7 days after injury. AGH-194 was intraperitoneally (i.p.) administrated (5, 10, 20 mg/kg) and then behavioural tests were assessed (reaction to mechanical stimuli in von Frey test and to thermal stimuli in cold plate test). The involvement of brain 5-HT system in thermoregulation is known so we investigated body temperature after AGH-194 injection via rectal procedure. AGH-194 diminished symptoms of neuropathy and observed analgesic effect was time- and dose-dependent. Moreover, AGH-194 enhanced morphine analgesic effects. We did not observed any influence of AGH-194 on body temperature. Here, we have shown that newly synthesized 5-HT_7 agonist (AGH-194) diminished neuropathic pain symptoms and potentiate morphine analgesia, which is clinically relevant. AGH-194 posses favorable drug properties, e.g. metabolic stability, BBB permeation, water solubility, lack of influence on body temperature and low cytotoxicity in human cell lines, therefore, it can be considered as promising candidate for further therapy.

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STEP FORWARD IN SEARCH FOR NOVEL HISTAMINE H_3 RECEPTOR LIGANDS: 4-PYRIDYL-PIPERAZINE DERIVATIVESWITHPROMISINGMULTIDIRECTIONAL PHARMACOLOGICAL ACTIVITY

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Histamine H_3 receptors (H_3R) are constitutively active G-protein coupled receptors mostly expressed in CNS. Interaction with these receptors results in modulation of histamine levels as well as that of other neurotransmitters. Therefore, blockade of these receptors might provide useful pharmacological target in treatment of many CNS-based diseases such as schizophrenia, Alzheimer and Parkinson's disease, obesity, narcolepsy and attention-deficit hyperactivity disorder (ADHD) [1], also as dual acting ligands [2].

Undoubtedly, the imidazole ring replacement with other heterocyclic moieties was a milestone in the search for new histamine H_3R ligands. A piperazine moiety is such a replacement, being a significant versatile scaffold in rational drug design for most of the GPCR ligands.

Based on the results of the research so far, it is assumed that the 4-pyridylpiperazine moiety in the basic part of the compound determines their high affinity and selectivity for human H_3R. A position of nitrogen atom in an aromatic ring attached to piperazine moiety has turned out to be a key structural element for suitable interaction with its biological target. Recent studies had shown, that Pitolisant might reduce body weight in obese mice [3]. To determine potential anti-obesity properties of selected compounds, preliminary pharmacological in vivo tests have been performed. In these studies, the influence on body weight, food and water intake, glucose and triglyceride plasma levels, as well as spontaneous activity on rats in the model of excessive eating of preferential feed was tested. Pharmacological in vivo test results of compound KSK3 clearly indicate that it may affect the amount of calories consumed, thus act as an anorectic compound. Likewise, its protective action against hyperglycemia and the development of overweight has been shown.

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HISTAMINE H_3 RECEPTOR LIGANDS WITH MONOAMINE OXIDASES INHIBITORY ACTIVITY

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According to data from the World Health Organization (WHO), the human population is aging all over the world [1]. As a result of this process, an increase in the incidence of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases is observed. The basis of these diseases is gradual and irreversible, progressive degradation of neurons leading, among others, to significantly reduce the ability of the human body to maintain homeostasis in response to adverse environmental factors. Unfortunately, no clear strategies for degenerative diseases have been so far developed due to a lack of full knowledge of their pathogenesis. On the other hand, compounds acting through 2 or more biological targets common to one disease, might increase the therapeutic effect.

Histamine H_3 receptors (H_3R), constitutively active G-protein coupled receptors mostly expressed in CNS, regulate histamine levels as well as that of other neurotransmitters such as ACh, NA, 5-HT etc. Therefore, blockade of these receptors might provide useful pharmacological target in treatment of many CNS-based diseases [2, 3], also as dual acting ligands [4-6]. Moreover, recent data show promising MAO-A/MAO-B reverse inhibition properties of know histamine H_3R antagonist Ciproxifan [7].

In this study, histamine H_3R ligands obtained in our group were tested against MAO-A and/or MAO-B. Following molecular docking studies to desired biological targets were undertaken in order to visualize and better understand possible mechanisms of action.

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EFFECTS OF PHARMACOLOGICAL MODULATION OF THE HISTAMINE H_3 RECEPTOR ON THE PROCESS OF NOCICEPTION IN NEUROPATHIC PAIN

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Evidences have shown that histamine system might be a key modulator of neuropathic pain. Especially interesting target is histamine H_3R receptor, while its expression has been reported in nociceptive transmission pathways. The aim of our study was to determine the analgesic effects of novel H_3R (E-162; 1-(5-(naphthalen-1-yloxy)pentyl)piperidine) antagonist in preclinical model of neuropathy (CCI, chronic constriction injury) in mice. We investigated the E-162 action on mechanical (von Frey) and thermal (cold plate, tail flick) stimuli in naive and CCI-exposed (7 days after injury) mice. Differences between males and females in pain perception have been suggested, therefore, we analysed sex-dependent action of E-162. We investigated the influence of antagonist on motor coordination (RotaRod). We also carried out an experiments to investigate the influence of E-162 on morphine analgesia. Moreover, to evaluate the mechanism of E-162 action, we analysed the participation of spinal H_1R in tested antagonist effects. We revealed analgesic potency of E-162 in naive mice (tail flick). E-162 also attenuated nociception in neuropathic males, which was reversed after pyrilamine (H_1R antagonist) pretreatment. Antagonist also potentiated morphine analgesia. Moreover, it produced prolonged analgesia in neuropathic females. Our work provides the first evidence for the analgesic potency of novel H_3R antagonist and its beneficial properties for morphine effectiveness during neuropathy. We have also evaluated sex-dependent differences in analgesic effects of E-162. Our results demonstrated that H_3R is intimately involved in nociceptive transmission during neuropathy and targeting H_3R can potentiate morphine analgesia, which is consistent with multimodal pain therapy.

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MONOAMINE OXIDASE B INHIBITION OF NOVEL ANALOGS AND DERIVATIVES OF 1-[3-(4-TERT-BUTYL-PHENOXY)PROPYL] PIPERIDINE

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Histamine H_3 receptors (H_3Rs) are one of the four known histamine receptors. They are mostly expressed in CNS and regulate the release of histamine and other neurotransmitters (e.g., acetylcholine, noradrenaline, dopamine, serotonin). Antagonists/inverse agonists of H_3R are interesting in the search for new drugs for CNS diseases [1]. Monoamine oxidase A and B are two izoenzymes that catalyze deamination of molecules like dopamine and serotonin in the presence of oxygen [2]. Monoamine oxidase B (MAO-B) is known for its crucial role in neurodegenerative diseases. Therefore, dual targeting (H_3R and MAO-B) can be interesting approach for treatment of neurodegenerative disorders [3].

1-[3-(4-*tert*-Butyl-phenoxy)propyl]piperidine (DL-76) is a non-imidazole H_3R antagonist with high potency both *in vitro* (hH_3R K_i = 22 nM) and *in vivo* (ED_{50} = 2.8 mg/kg p.o) [4]. In this study, we investigated a group of novel analogs and derivatives of DL-76 for the activity at human MAO-B, and selected of them for possible inhibition of human MAO-A. The inhibitory potency at toward human MAO-B was evaluated using Amplex Red® Monoamine Oxidase kit (LifeTechnologies). Inhibition activity was measured in the presence of the substrate, p-tyramine (200 μM), and was compared to the activity of the inhibitors: MAO-B pargyline and rasagiline; MAO-A clorgiline. Moreover, the most active inhibitors were tested for inhibition kinetics, and modality.

In the group of tested compounds, we have found H_3R ligands that can inhibit MAO-B in submicromolar concentration range. These structures do not show MAO-A inhibition. Structure-activity relationship analysis will be helpful for a further research in the area of dual targeting H_3R and MAO-B ligands.

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ASSESSMENT OF SELECTED PARAMETERS OF IMMUNE RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES IN THE PRESENCE OF GRAPHENE

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Potential cytotoxicity is a key issue decisive in use of nanomaterials in biomedical patents. The aim of the study was assessment of graphene cytotoxicity by examination its impact on activation of myeloperoxidase, caspase 9 and γ-H2AX. For the study were used three different forms of graphene, A02, A03 and A04 and graphene deposited on silicon thin slice ($GrSiO_2$). Lymphocytes were incubated with graphenes each at a different doses and concentration. Cells viability was measured using the CellTiter-Blue®Test. The lymphocytes morphology after exposure to graphenes were examined under the scanning electron microscope. Measurement of quantity of myeloperoxidase (MPO), caspase 9 and γ-H2AX was carried out by ELISA. The highest number of living cells was found in the case of graphene A04, the lowest in the case of graphene A02. On the basis of analysis of lymphocytes morphology, no notice was given that their structure was changed. The highest concentration of MPO and γ-H2AX was found in the group of cells incubated with A02 and A03. The highest level of caspase 9 was observed in the population of cells incubated with A04, the lowest with $GrSiO_2$. Graphene A02 (AFT 8 nm) and A03 with small nanoparticles (AFT 12 nm) has shown to be much more toxic than A04 with large nanoparticles (AFT 60 nm). SEM analysis showed strong adsorption of the cells to the surface of nanoparticles which may indicate chemotactic impact of graphene on the cells. In the case of caspase 9, A04 showed the highest activation ability. In the case of analysis of MPO and γ-H2AX level, A03 and A02 had the greatest impact on its quantity. Probably, small nanoparticles strongly activate oxidative stress which, in turn, causes the disturbance of the cell DNA structure. A04, as well as $GrSiO_2$, turned out to be the safest form of graphene in relation to lymphocytes. It can therefore be concluded that large-size graphene and graphene deposited on solid are the most biocompatible forms in regard of tested cells.

SERUM CONCENTRATIONS OF OPG AND RANKL IN RHEUMATOID ARTHRITIS IN DIFFERENT BIOLOGIC THERAPIES

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Osteoprotegerin (OPG) is a soluble decoy receptor which blocks osteoclast differentiation and activation by neutralizing the receptor activator of NF-κB ligand (RANKL). The balance between RANKL, which stimulates osteoclastogenesis and osteoclastic activation, and its physiological antagonist OPG plays a critical role in the regulation of bone resorption in rheumatoid arthritis (RA).

The aim of the study was to examine the impact of various drug therapies in RA on the bone turnover activity markers, i.e. sRANKL and OPG.

A group of 125 patients (pts) with RA and a control group of 42 healthy people have been qualified to the study. All patients fulfilled the American College of Rheumatology (ACR 2010) criteria for RA. RA group was divided into several subgroups. First group included 39 RA pts on Leflunomide. Second group included 49 RA pts on Methotrexate (MTX) and etanercept (First line biologic therapy). Third group included 16 RA pts on MTX and adalibumab, golibumab, infliksimab (Second line biologic therapy). Fourth group included 16 RA pts on MTX and antiCD20 or anti IL6 (Third line biologic therapy). Fifth group included 44 RA pts on Disease-modifying antirheumatic drugs (DMARD's), 39 of them were included into Leflunomide therapy, also with intolerable toxicity to MTX. Estimated research period was 12 months. Average age of participants was 54 (22-79 years). All pts have been examined based on DAS28 before and after 90 days of therapy. The blood samples for bone markers RANKL and OPG levels were measured by ELISA after 90 days therapy. Bone erosions in hands and feet were evaluated by Larsen methods. Dexa scan of femoral neck was performed.

All subgroups of RA pts were compared in respect of markers of osteoporosis. The individual groups of pts do not differ from each other in the following parameters: the radiological destructions of the disease, organ damage, presence of anti-CCP and RF, the average use of GKS, and co-occurrence with osteoporosis and osteopenia. The above-mentioned groups of pts were relatively homogeneous.

In all groups of RA pts treated with various therapies decreased level of sRANKL/OPG has been observed compared to DMARD therapy (Leflunomide to DMARD's – p=0.06, First line biologic therapy to DMARD's – p=0.05, Second line biologic therapy to DMARD's- p=0.04, Third line biologic therapy to DMARD's – p=0.001. Additionally in the RA group treated with anti CD20 and anti IL6 therapy serum OPG level was significantly higher than in other group (p=0.003). Furthermore, serum sRANKL level was reduced in the third-line therapy compared to DMARD's (p=0.003).

In conclusion our findings indicate that both OPG as well as sRANKL help with evaluation of treatment effectiveness in RA and are useful parameters in daily clinical practice.

THE OPPOSITE EFFECTS OF LEPTIN AND ADIPONECTIN ON MAST CELLS INFLAMMATORY RESPONSE

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There is growing evidence indicating that adipocytokines, biologically active adipose tissue-derived molecules, have a significant impact on the functioning of the innate immune system. They are one of the major components of the humoral constituent network. Considering the role of adipocytokines, they may be recognized as mainly pro-inflammatory factors, like leptin, or an anti-inflammatory, such as adiponectin. As mast cells (MCs) are the key players in the course of immunological and inflammatory processes, it seems to be of great importance to determine the direct effect of leptin and adiponectin on MC activity.

The aim of the study was to determine the influence of leptin and adiponectin on MC inflammatory activity.

Experiments were conducted in vitro on mature connective tissue MCs isolated from rat peritoneal cavity. The effect of leptin and adiponectin on MC degranulation (histamine release assay), cysteinyl leukotriene (cysLTs), IL-10 and CCL2 synthesis and release (qRT-PCR and ELISA technique) was estimated.

We found that leptin, but not adiponectin, stimulates MCs to degranulation and histamine release. A significant histamine secretion occurred within 5 minutes in response to leptin stimulation. MCs also synthesize and release substantial amounts of cysLTs following exposure to leptin, and adiponectin does not activate these cells to cysLT production and release. Moreover, MC stimulation with leptin, but not adiponectin, increases CCL2 mRNA and protein expression. In turn, adiponectin, but not leptin, upregulates IL-10 mRNA and protein expression in MCs.

Collectively, we observed that leptin promotes the pro-inflammatory response of MCs (via histamine, cysLT, and CCL2 generation and release), whereas adiponectin induces anti-inflammatory IL-10 synthesis and release from those cells. Hence, leptin and adiponectin are engaged in the inflammatory processes involving MC participation.

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THE EFFECT OF FUNGAL ANTIGENS ON MAST CELL ACTIVITY

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Mast cells (MCs) are long-lived resident tissue cells numerously distributed throughout the body, and they take part in various processes, inter alia innate immune response. MC activity against bacteria and viruses is well known. More and more data suggest that MCs also take part in anti-fungal immune response, however available data are scarce. Main antifungal antigens are zymosan and glucan, which constitute an element of a fungal cell wall.

The study aimed to determine the influence of cell wall components zymosan and glucan, on MC inflammatory activity.
Mature connective tissue MCs were isolated from rat peritoneal cavity. The effect of zymosan and glucan on MC degranulation (histamine release assay), cysteinyl leukotriene (cysLT) release (ELISA assay), and cell migration (Boyden chamber) were estimated.

We have noticed that zymosan causes significant histamine release after 30 minutes of stimulation. Impact of glucan on histamine release is even more pronounced since substantial levels of histamine secretion occurred after 5 minutes of stimulation. We have also shown that MCs synthesize and release significant amounts of cysLTs following exposure to zymosan and glucan. Moreover, we have stated that both fungal antigens may affect MCs migration.

Fungal antigens zymosan and glucan may directly activate MCs and promote their inflammatory response.

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EXPRESSION OF THE FUNCTIONAL LEPTIN RECEPTOR ON THE MAST CELLS

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Leptin, the adipose tissue-derived product of the obese (*ob*) gene, is known to function as the hormone of energy expenditure. It has also been established that leptin regulates immune and inflammatory processes. However, the available data relating to the leptin receptor (Ob-R) on mature mast cells (MCs), and consequently leptin significance in the modulation of MC activity within the tissue, are limited.
The aim of this study was to establish constitutive and leptin-induced Ob-R expression by tissue MCs.

For determination of the constitutive and leptin-induced expression of Ob-R on mature connective tissue rat MCs, immunohistochemistry, flow cytometry, and confocal microscopy were used. Leptin-induced MC histamine release was measured spectrofluorometrically.

Tissue MCs express Ob-R constitutively and this receptor is located on the cell surface and intracellularly. We documented that leptin influences Ob-R expression on peritoneal MCs. At lower concentrations, it causes Ob-R expression increase both at the cell surface and in the cell interior. MC stimulation with higher concentrations of leptin results in a decline of Ob-R from the cell surface and significant enhancement of this receptor not only in the nuclear region but also in the endoplasmic reticulum. Moreover, leptin-stimulation causes histamine release from MCs, and JAK2 and ERK inhibitors significantly diminish this MC response.

Tissue MCs express specific functional receptor for leptin. This adipokine by itself affects Ob-R expression level in these cells. Thus, one can be assumed that leptin regulates MC activity within tissues.

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SHORT-TERM HYPOXIA REGULATES LAD2 MAST CELL ADHESION TO FIBRONECTIN THROUGH INTEGRIN $\alpha5\beta1$ ACTIVATION

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Mast cells (MCs) play a significant role in the initiation, amplification and resolution of the inflammatory response. The state of hypoxia can lead to the development of an inflammatory reaction, as well as the ongoing inflammatory process can trigger hypoxia conditions. Binding of fibronectin with various cells of the immune system takes place with the participation of integrin receptors that are regulated by the cell in the inside-out signaling.

Human LAD2 MCs were cultured for 3 days under standard (21% O_2) or hypoxic conditions (1% or 5% O_2). Next cells at a density of 50 000 cells/well were incubated for 1 h under standard or hypoxia conditions on 96-well plate coated with fibronectin. Following removal of non-adhering cells acid phosphatase was used as a marker for determining the percentage of adhering cells. We investigated the expression of surface receptors, the expression of genes encoding integrins and adhesion assay using the RGDS peptide as well as HIF-1α proline hydroxylase inhibitor (IOX2) and FAK kinase inhibitor (PF-573228).

LAD2 MCs exhibit high spontaneous adhesion to fibronectin which grows stronger when the cells are exposed to reduced O_2 concentration. Both short (1 hour) and longer (3 days) hypoxia treatment causes a significant increase in adhesion to fibronectin. RGDS peptide contributes to a significant reduction in adhesion under 21% O_2, while in hypoxia this effect is not as strong. Expression of surface integrin $\alpha5\beta1$ revealed no differences between cells incubated under standard conditions and those in hypoxia. Adhesion assay with the anti-$\alpha5\beta1$ antibody significantly inhibits adhesion. HIF-1 alpha pathway inhibitor IOX2 did not affect the adhesion of LAD2 MCs to fibronectin. The Focal adhesion kinase inhibitor PF-573228 decreased adhesion regardless of the conditions.

Short term hypoxia modulates MC adhesion to fibronectin increasing integrin $\alpha5\beta1$ dependent adhesion. The probable mechanism responsible for increasing adhesion to fibronectin under hypoxia conditions should be sought primarily in the inside-out integrin signaling pathway.

EXPRESSION OF GENES ASSOCIATED WITH HISTAMINE RECEPTORS IN PATIENTS WITH PSORIATIC ARTHRITIS TREATED WITH ANTI-TNF BIOLOGICAL THERAPY

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Adalimumab, a biological anti-TNF drug, has positive effects in the treatment of psoriasis. However, there is still a lack of information on the systemic activity of this drug with prolonged use.

The aim of the study was to evaluate systemic changes in the expression profile of genes associated with histamine receptors in patients with psoriatic arthritis treated with adalimumab for 4 years.

The study material consisted of blood samples of patients suffering from psoriatic arthritis treated with adalimumab. The drug was administered subcutaneously at a dose of 40mg every 2 weeks. Blood samples were collected before administration of the drug and 2 hours after its administration. The control group consisted of blood samples taken from healthy people. The analysis of the expression profile of genes associated with histamine receptors was carried out using HG-U133A 2.0 oligonucleotide microarrays (Affymetrix, Santa Clara, CA, USA). Statistical analyzes were performed using the PL-Grid infrastructure.

In the study, there were no statistically significant differences in HRH1 and HRH2 receptor transcriptional activity, while significant change of expression of 7 genes encoding proteins associated with HRH1 activity was demonstrated, including GNG10 and GNG5 (overexpression) and ITPR3, PLCG1, ITPR1, PRKCH, PRKACA (underexpression).

In contrast, 5 genes associated with HRH2 were observed, including 3 in overexpression (GNAS, GNG10, GNG5) and 2 in underexpression (PRKACA, PRKACB). After administration of the drug in the case of HRH1, the expression of GNG10 increased, whereas in the case of HRH2 – GNG.

Analysis of concentration profile of 22 277 mRNAs showed that among the genes associated with biogenic amine activity statistically significant differences were observed only in the genes related to histamine activity, which indicates that genes associated with histamine receptors may be related to the observed improvement in the clinical condition of patients under effects of anti-TNF targeted therapy.

THE RELATIONSHIP BETWEEN HISTAMINERGIC SYSTEM AND IL12/23 SIGNALING PATHWAY IN NHDF CELL CULTURE AFTER ADALIMUMAB TREATMENT

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The interaction between biogenic amines and amine signaling pathways is an interesting topic. IL-12 and IL-23 activate the JAK/STAT cascade. Adalimumab is a fully monoclonal anti-TNF antibody. The neutralization of TNF-α through its binding by the adalimumab will also influence the expression pattern of other cytokine, biogenic amine and activated by the signal paths. The better understanding the relationship between different signaling cascades is a key step to more effective therapy.

The aim of this research was to evaluate the impact of adalimumab on the expression profile of genes associated with the histaminergic system and IL12/23 signaling pathway and finding a relationship between them in Normal Human Dermal Fibroblast cells stimulated with adalimumab for 2, 8, 24 hours. Control cells were not treated with adalimumab.

The expression profile of analyzing genes was appointed with the use of oligonucleotide microarrays HG-U133A (Affymetrix). Our work was supported in part by PLGrid Infrastructure.

Among 22283 mRNAs, 213 are connected with histaminergic system and IL12/23 signaling pathway. It can be observed 73 mRNAs differentiating NHDFs cultures with adalimumab from control ($p<0.05$). As the cell exposure time to anti-TNF medicine increased, the number of differentiating mRNAs was reduced (2 h vs C= 54 mRNAs; 8 h vs C= 32 mRNAs; 24 h vs C= 4 mRNAs). After 2 and 24 h, overexpression of HRH1 and reduce of transcriptional activity of IL12A can be observed . Regardless of time the expression of STAT1, JAK2, JAK3 are higher than in the control. The common gene for histaminergic system and IL12/23 pathway is CAV1 (for 2 and 24h of NHDF exposure to adalimumab).

The influence of adalimumab to expression profiles of histamine receptor and IL12A are opposite. This confirms the complexity of the interactions between signaling cascades and the effect of adalimumab on them. CAV1 seems to be the link between the histaminergic system and the IL12/23 pathway.

EVALUATION OF CHANGES IN THE EXPRESSION PROFILE OF GENES INVOLVED IN MITOPHAGY IN COLORECTAL CANCER

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Mitophagy is a type of autophagy involved in the removal of damaged mitochondria. In addition, it prevents the accumulation of abnormal mitochondria and mitochondrial cytotoxic metabolic products. Impaired mitophagy leads to accumulation of damaged mitochondria, which causes uncontrolled growth of reactive oxygen species, mutations in mitochondrial DNA, disturbances in energy management and, as a result, cell death.

The aim of the study was to assess the relationship between the expression profile of genes involved in the regulation of mitophagy and the stage of colorectal cancer (CSI-CSIV).

276 mRNAs associated with the activity of mitophagy were analyzed. The expression profile was determined using HG-U133A microarrays (Affymetrix, Santa Clara, CA). Determination of differentially expressed genes was carried out using PL-Grid Infrastructure (http://www.plgrid.pl/).

Based on a statistical analysis, it was found that out of 276 mRNAs of genes associated with mitophagy, 48 mRNAs were significantly differentially expressed in adenocarcinoma compared to control. The obtained results showed that the number of mRNAs differentiating each cancer stage from the control was as follows: CSI vs. control, 6; CSII vs. control, 11; CSIII vs. control, 18; and CSIV vs. control, 20 ($p<0.05$). Observed differences in the transcriptional activity of genes associated with mitophagy in colorectal cancer indicate that the higher the clinical stage of the cancer, the lower the activity of mitophagy.

DIVERSITY OF EXPRESSION OF GENES ENCODING CASPASES DEPENDING ON THE CLINICAL STAGE OF COLORECTAL CANCER

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Colorectal cancer is one of the most frequent cancer type occurring in the world. Recent studies have shown that melatonin therapy can be a new treatment strategy for this type of cancer. Melatonin is a hormone synthesized in the human pineal, however it is also produced in considerable amounts by mammal gastric tract. Melatonin shows oncostatic properties which come from its ability to inhibit angiogenesis and metastasis and promote apoptosis. Cucina et al. suggest two separate ways of melatonin proapoptotic activity: early TGF-β1 response, independent of caspases and late apoptotic TGF-β1 process in which caspase-7 is probably activated as a final effector.

The aim of the study was to evaluate the variation in the expression level of genes encoding caspases depending on the colorectal cancer stage.

Material consisted of 24 cancerous tissue samples and 24 noncancerous (marginal) tissue samples obtained from patients with colorectal cancer in clinical stages: I (CS-I), II (CS-II), III (CS-III) and IV (CS-IV) by surgical intervention. In addition, tissues obtained during colonoscopy evaluated as noncancerous were used as a control. Expression level of genes coding caspases was evaluated with the use of oligonucleotide microarray technique (HG-U133A, Affymetrix).

25 ID mRNAs for caspases genes were analyzed. Overexpression of apoptotic initiator caspase-2 gene was observed in every cancer sample in comparison to control. However, statistical significant changes was observed only in case of CS-I. Expression of caspases coding genes doesn't show relation to clinical stage of colon cancer. However, colon cancer characterizes itself with caspase-2 overexpression, what can lead to apoptosis initiation through the cell-extrinsic pathway.

MELATONIN AND CIRCADIAN RHYTHM REGULATION IN ENDOMETRIAL CANCER

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Studies on the correlation between melatonin secretion and potential carcinogenesis have been conducted since the 1970s. In 2017, the Nobel Prize was awarded for explaining and describing the mechanisms of regulation and control of circadian rhythms. Melatonin is involved in many biological processes and one of the best known properties of melatonin is regulation of the circadian rhythm. Its disruption may result in the appearance of pathology in various tissues.
The aim of the study was to select mRNAs associated with melatonin and involved in circadian rhythm regulation in normal endometrial tissue and G1-G3 endometrial cancer.

The study enrolled 27 patients who underwent hysterectomy in Department of Gynecology Silesian University Hospital in Katowice. 24 patients with endometrial cancer in 3 degrees of differentiation (G1-G3; the study group), and 3 patients without endometrial cancer qualified for hysterectomy due to the pathologies of the uterus such as its prolapse and uterine fibroids (the control group). All 27 samples (control, 3; G1, 7; G2, 11; and G3, 6) were selected for microarray analysis with the use of HG-U133A arrays (Affymetrix, Inc., Santa Clara, CA, USA). Statistical analysis was performed using GeneSpring 13.0 and PL-Grid platform.

The study demonstrated that 129 mRNAs representing genes associated with melatonin and circadian rhythm regulation were significantly differentially expressed in endometrial cancer compared to control. We have observed that CLOCK participating in the regulation of circadian rhythms is silenced and differentiates G2 from the control ($p<0.05$, FC = 1.8877153)

The study showed that melatonin can potentially play a role in the regulation of circadian rhythm in endometrial cancer. Silencing gene encoding CLOCK proteins, positive regulators controlling the expression of negative regulators, may cause circadian rhythm disruption, and thus may be related to the pathological changes observed in endometrial cancer.

MEMBRANE AND NUCLEAR MELATONIN RECEPTORS IN ENDOMETRIAL CANCER

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Melatonin is a hormone synthesized in the pineal gland and secreted directly into the blood, as well as locally by multiple cells and organs such as glial cells, retinal cells, testes, ovaries, gastrointestinal tract, lymphocytes. In humans, two membrane receptors with high affinity for melatonin have been identified: MT1 and MT2 receptors, located in various tissues, including the endometrium. Melatonin can also penetrate into the cell and associate with the RZR/ROR receptors in the cell nucleus.

The aim of the study was to evaluate the expression profile of membrane and nuclear melatonin receptors in normal endometrial tissue and endometrial cancer.

The study group involved 24 women with endometrial cancer in 3 histopathological grades (endometrial adenocarcinoma G1, G2 and G3). The control group consisted of 3 patients with uterus diseases such as uterine prolapse and uterine fibroids. All patients underwent hysterectomy in Department of Gynecology Silesian University Hospital in Katowice. Molecular analysis was carried out in Department of Molecular Biology at the Medical University of Silesia. Microarray analysis on all 27 samples (control, 3; G1, 7; G2, 11; and G3, 6) was performed with the use of GeneChip® Human Genome U133A arrays. Statistical analysis was performed using GeneSpring 13.0 and PL-Grid platform.

The study showed no significant changes in the expression profile of genes encoding nuclear melatonin receptors in endometrial cancer compared to the control. In the case of MT1 and MT2 membrane receptors, individual variability results in a large spread of results and, despite clear differences, no statistical significance is observed. Literature indicates the potential use of melatonin in the treatment of various types of cancers. Our study has shown that this type of modern therapy using the potential of melatonin may not be justified in the treatment of endometrial cancer, however, further research may be necessary.

POTENTIAL CROSSTALK BETWEEN MELATONIN AND EPITHELIAL MESENCHYMAL TRANSITION IN ENDOMETRIAL CANCER

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The epithelial mesenchymal transition (EMT) is a process of molecular reprogramming during which a cell with an epithelial phenotype is converted to a mesenchymal cell. As a result, polarized and immobile cells acquire new structural and functional features, which leads to increased mobility and invasion. During the neoplastic process, the initiation of EMT leads to cancer progression and metastasis. Melatonin is a hormone that displays oncostatic activity through a number of mechanisms, including modulation of oncogenes expression, antioxidant and anti-angiogenic effects. Studies also suggest that melatonin has anti-invasive and anti-metastatic effects, which is why there is a great interest in its use in the treatment of cancer.

The aim of the study was to evaluate the expression profile of genes associated with melatonin activity and epithelial mesenchymal transition in endometrial cancer.

The study consisted of 27 women: 24 with endometrial cancer (study group) and 3 without neoplastic changes (control group). The study group was further divided according to the degree of histopathological differentiation: G1, 7; G2, 11; and G3, 6. The transcriptional activity of selected genes was assessed with the use of HG-U133A oligonucleotide microarrays (Affymetrix, Santa Clara, CA). Statistical analysis was performed using GeneSpring 13.0 and PL-Grid platform.

The study demonstrated that there were significant changes in the expression profile of 129 mRNAs representing genes associated with epithelial-mesenchymal transition and melatonin activity, e.g. WNT2, WNT4, WNT5A, MUC1, MUC5AC ($p<0.05$, $FC>2$ or $FC<-2$).

The dominant signaling pathway involved in EMT in endometrial cancer is Wnt, and the lack of changes at the level of melatonin suggests that it does not participate in this process.

ASSESSMENT OF PROGRESS IN TREATMENT OF CHRONIC PRESSURE ULCERS USING METALLOPROTEINASE AND CYTOKINE GENES EXPRESSION

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Matrix metalloproteases (MMPs) are a family of proteases that can be stimulated through histamine in keratinocytes [1]. Histamine can influence skin fibroblast differentiation and play a role in fibrotic events in wound healing process. TGF-β induces downregulation of H_1R on fibroblasts leading to a balance between other molecules. MMP-2 and MMP-9 can activate transforming growth factor beta 1 (TGF-β1), which acts as a negative autocrine growth factor and controls proliferation, differentiation in many cell types. This can indicate that these molecules play important role in skin wound healing processes together with histamine [2].

Bedsores, from the physiological point of view, are chronic wounds, which are not subject to the process of easy healing. That constitutes a serious health problem which concerns millions people in the world [3]. To the problem are not engaged only physicians, physiotherapists or pharmacists, but also scientists giving a new solutions to support the healing process. However, long-term treatment requiring use of various pharmaceutical preparations and expensive stimulants not always bring the desired results. Therefore, a new therapeutic methods which do not require large financial outlays and can be used at home by the patient or his nearby, are still being of need. Many cellular processes take place during wound healing and are coordinated by such molecules as: cytokines, chemokines, growth factors or proteases. These factors are conditioning wound healing, and acting through autocrine and paracrine mechanisms, which regulate migration processes, proliferation and cell differentiation.

Thus, the treatment progress of chronic pressure ulcers using electrostimulation methods and pharmacotherapy was analyzed by evaluating the expression of MMP-2, MMP-9 and TGF-β1 genes and their relationship to each other during three periods of medication.

The results show that the used genes changed their expression during the treatment. When evaluating the progress of treatment, a decrease in expression of metalloproteinases in the absence of treatment, whereas between a two and four-week of therapy with cathodic electrostimulation their increase was observed. We can conclude that expression of these genes may be helpful in the assessing the treatment progress of the chronic bedsores by used methods.

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RESPONSE OF SELECTED CELLULAR PARAMETERS OF THE IMMUMOLOGICAL SYSTEM IN THE COURSE OF PROTON PUMP INHIBITORS THERAPY

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Proton pump inhibitors (PPIs) are a group of drugs commonly used in the treatment of gastrointestinal diseases associated with excessive HCl secretion in the stomach. The introduction of PPIs into gastroenterological practice brought a breakthrough in the treatment of gastric and duodenal ulcers, gastroesophageal reflux disease (GERD), helped eradicate Helicobacter pylori infections, as well as prevent from gastrointestinal (GI) damage due to nonsteroidal anti-inflammatory drugs (NSAIDs), or pharmacotherapy used in the treatment of upper GI bleed of non-variceal origin, and functional dyspepsia. Inhibition of acid secretion in the stomach is achieved by inhibiting H, K-ATPase enzyme, commonly referred to as the proton pump.

Proton pump inhibitors increase gastric pH via a specific mechanism, which affects the absorption of certain drugs and nutrients, i.e. iron, vitamin B12, magnesium, and calcium. In addition, altered gastric pH is of clinical relevance, as it increases the risk of infection both within the gastrointestinal tract and the respiratory system.

The aim of this study was to evaluate the effects of proton pump inhibitors on the selected immune parameters including phagocytic activity and the ability to synthesize reactive oxygen compounds (respiratory burst) of the immune cells, i.e. monocytes and neutrophils in the patients on PPI treatment. The study was conducted on a group of 47 patients, aged 22 to 79 years, treated for GERD, functional dyspepsia and chronic gastritis in the Out-Patient Clinic of Gastroenterology, Independent Public Clinical Hospital No 4 in Lublin, the Out-Patient Clinic of Gastroenterology, Provincial Hospital in Tarnobrzeg, and the Center of Gastroenterology and Nutrition "KOSKOMED" in Tarnobrzeg in the period from 28 October, 2013 to 29 December, 2014.

In the group of patients on pantoprazole in the doses of 20 mg or 40 mg daily for 28 days, 5-ml samples of peripheral blood were collected into heparin-coated tubes. The blood was collected at three intervals:

- prior to treatment,
- on day 7 of treatment,
- on day 28 from the start of treatment.

The phagocytic activity and phagocyte aerobic metabolism of neutrophils and monocytes was evaluated on the FACSCalibur flow cytometer equipped with argon laser, 488 nm (Becton Dickinson, USA).

The results let formulate the following conclusions:

1. The use of proton pump inhibitors results in altered immune response in the treated patients.
2. Changed immune response in the patients on proton pump inhibitors is manifested as impaired phagocytosis and the 'respiratory burst' in the neutrophils and monocytes.
3. The said immune dysfunctions occur at early stages of PPI treatment, i.e. in the first week of the drug administration, and normalize after 4 weeks of treatment.
4. The disorders of the selected immune parameters are not related to patient age or gender.

GRAPHENE INTERACTIONS WITH HUMAN ENDOTHELIUM

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The discovery of graphene boosted an interdisciplinary research based on its unique properties and possible applications in biomedicine. Multiple scientific reports concerning graphene interactions with adherent cells showed the potential use of graphene as a biomaterial in tissue engineering.

Graphene samples used in the study were synthesized by chemical vapor deposition method. Raman Spectroscopy confirmed the presence of high quality graphene monolayer, especially on the glass samples, which were further taken to the analysis. The projects assumptions include comprehensive studies on the graphene impact on human endothelial cells isolated from umbilical vein through enzymatic digestion procedures. HUVEC's culture provide an ideal model to study many aspects of endothelial function including physiological healing and processes present after stent implantation. Multidirectional studies include endothelial cell morphometric study, viability and proliferation assays, as well as IE and IF analysis concerned with the expression of CX3CL1 chemokine and its receptor.

The research proved that graphene monolayer used in the experiments support endothelial cell growth and viability. Image analysis did not show any detectable changes in cell morphology, impairment in cell adhesion and nuclear disturbances present in apoptotic cells. Studies revealed that graphene directed the cell growth, which was confirmed by the increased expression of stress fibers and presence of cellular extensions. After 24 hours, graphene monolayer transferred on the glass did not significantly affected cell viability but increased cell number. In the subsequent days, the degree of proliferation decreased, and never reached the value obtained after cell activation with TNF-α. Graphene used as a growth substrate did not affected endothelial cell immune response associated with the expression of chemokine CX3CL1.

Presented results pre-assessed the possibility of graphene to be used as a biomaterial in the production of intravascular devices. Pristine graphene on the glass substrate favors cell growth giving the opportunity to fast and effective re-endothelialization. Graphene as a biomaterial would ensure proper and accelerated vascular healing and reduce already existing complications associated with the stent placement.

CHANGES IN SEROTONIN AND TNF-INDUCED PATHWAYS IN ADSC CO-CULTURED WITH LESC CELLS

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Corneal damage can be caused by many factors, including inflammatory diseases, and may result in limbal stem cells deficiency (LSCD). The treatment of LSCD is limited because of the deficit of donor tissues. Adipose derived stem cells (ADSCs) could be differentiated into many types of cells and one of the possible strategy for LSCD treatment is the differentiation of ADSCs into limbal epithelial stem cells (LESCs). The possible molecular mechanism underlying ADSC differentiation is stimulation of changes in the serotonin (5-hydroxytryptamine, 5-HT) and Tumor Necrosis Factor (TNF)-induced pathways. Serotonin modulates many physiological functions and may act as a mitogen for stem cells. Moreover, 5-HT may modulate pathways induced by TNF. Through 5-HT_{2A} receptor serotonin can block TNFR1-induced pathways, especially NFκB pathway. The final result of crosstalk between these two pathways is still unclear.

The aim of this study was to assess the changes in transcriptomes of genes related to serotonin and TNF-induced pathways in ADSC co-cultured with LESC.
Differentiation of human ADSCs into the corneal epithelium was induced by co-culture with human limbal epithelial stem cells (LESCs). Total RNA was extracted using column-based method. The expression profiles of genes related to serotonin and TNF-induced pathways were determined using oligonucleotide microarrays (HG-U133A 2.0, Affymetrix). Differentiating genes were appointed with the use of GeneSpring 13.0 and PL-Grid platform ($p<0,05$ and $FC>3$).

From the set of genes related to TNF-induced pathways the strongest differences we found in ADSC cells co-cultured with LESC, comparing to control (LESC cells). The most upregulated genes were: TNFRSF1B encoding TNFR1, SMPD1, DUSP1 and MEF2A, the most downregulated were: BIRC5 and NRAS. Comparing to ADSC the most upregulated genes were: HSPA1A, HSPA1B and DUSP10. The most downregulated genes were: FOSB and DUSP2. From the set of genes related to serotonin-induced pathways the most differentiating genes were: MAP1A and LMOD1 (upregulated) and SNCA (downregulated), in comparison to control LESC cells. KCNK1 gene (upregulated) and HTR2A gene (downregulated) were changed in comparison to ADSC cells.

TNF-induced pathways may play pivotal role in the differentiation process of ADSC cells into LESCs.

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EXPRESSION OF MELATONIN RELATED GENES IN MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE AFTER CO-CULTURE WITH LIMBAL EPITHELIAL STEM CELLS

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Melatonin is an indoleamine, hormone which is mainly produced by the pineal gland. It is involved in regulation of various processes in organism as biological rhythm and immunity. Moreover, melatonin is known for its antioxidant properties. It acts through melatonin receptors MT1 and MT2, which are G protein-coupled receptors. However, there are also some studies which proved that melatonin is differentiation factor of stem cells. For example, melatonin promotes a differentiation into osteoblast or chondroblasts. Mesenchymal stem cell is a type of somatic stem cells with multipotent properties. They can differentiate into many types of cells from mesoderm, including osteoblasts, chondrocytes and adipocytes. There is a hypothesis that MSC can also differentiate into epithelial cells like corneal epithelial cells.
The aim of this study was to evaluate the expression of melatonin related genes in human ADSC (adipose derived stem cells) after co-culture with limbal epithelial stem cells.
ADSC (ADSC_D) were cultured 21 days in a presence of conditioned medium from limbal epithelial stem cells (LESC). Total RNA was extracted from cells using the High Pure RNA Isolation Kit (Roche Life Science). The expression profile of 700 genes related to activity of melatonin was appointed using oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Differentiating genes were selected by the use of GeneSpring software. ADSC cultured in standard medium served as control group (ADSC_C).
Analysis showed that 375 ID mRNA changed the expression with $p<0.05$. We observed 189 differentiating ID in ADSC_D compared to ADSC_C. It suggests that adipose derived stem cells after co-culture with LESC changed the expression of melatonin related genes and process of stem cells differentiation is probably associated with melatonin pathways.

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GENES RELATED TO HISTAMINE IN THE PROCESS OF ADSC DIFFERENTIATION AFTER CO-CULTURE WITH LESC

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Corneal blindness mostly originates from limbal stem cells deficiency. Limbal stem cells (LESC) are located at the corneal limbus (corneoscleral junction) from where they migrate and differentiate into the corneal epithelium. Limbal stem cells maintain proper efficiency and transparency of cornea and restore vision.
Adipose stem cells (ADSC) are mesenchymal stem cells derived from adipose tissue. Those cells can differentiate into multiple types of cells. Ability of these cells to gain corneal phenotype in the process of differentiation have not been confirmed yet. The use of ADSC instead of limbal stem cells would be a great source for the treatment of corneal blindness in case of the lack of donor tissue.
Histamine pathway plays multiple roles in human body. Also, the process of ADSC differentiation is regulated by thousands of genes. In this study a microarray analysis was performed to assess if there are any changes in the expression of genes related to histamine in ADSC after co-culture with LESC.
ADSC were cultured for 21 days in conditioned medium collected from LESC. After this period, total RNA was extracted using column-based method and microarrays were performed according to the manufacturers protocol. Differentiating genes were typed by the statistical analysis performed in Gene Spring software.
From 65 genes related to histamine, the expression of 31 genes significantly differed from other measured signals ($p<0.05$, ANOVA). Post-hoc analysis typed 11 differentiating genes in ADSC after co-culture with LESC in comparison to control group.
Co-culturing ADSC with LESC influence on the gene expression. Genes associated with histamine play a role in differentiation process of ADSC after co-culture with LESC.

This research was financed from the statutory research 2018, Medical University of Silesia in Katowice.

ACTIVITY BASED ANOREXIA IN FEMALE WISTAR RATS – A PILOT STUDY

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Routtenberg and Kuznesof (1967) observed that rodents have a tendency to self-starvation when exposed to restricted feeding and voluntary physical activity. As hyperactivity can be observed in a considerable subset of patients with anorexia nervosa (AN), animal models using the combination of a time-restricted feeding schedule and the access to physical exercise in a running wheel has been used to mimic features of AN.

The aim of the present study was to establish the model of activity-based anorexia (ABA) in our laboratory investigating food intake, running wheel activity and body weight in female Wistar rats. Animals weighing 170-220 g upon arrival were housed under controlled conditions and fed with standard chow (Labofeed B, Kcynia, Poland). The study was conducted in accordance with the institutional guidelines (65/2017). After an acclimatization period of 5 days, rats were randomly assigned to one of the following groups (n=8 each): (1) no extra activity + *ad libitum* feeding, (2) voluntary activity in a running wheel + a*d libitum* feeding, (3) no extra activity + restricted feeding and (4) ABA group: voluntary activity in a running wheel + restricted feeding. All cages contained environmental enrichment and bedding material, and were placed adjacent to each other to provide sight, acoustic and odor contact. Body weight, food intake and running activity were monitored until the body weight of the ABA group reached 75% of their initial body weight. Results were analyzed using ANOVA followed by a post hoc Tukey's test and expressed as mean ± standard deviation.

ABA rats showed a significant reduction in body weight in comparison to other groups ($p < 0.01$). ABA rats also showed a reduced daily food intake compared to other groups. Physical activity was significantly increased in ABA rats (24105.4 ± 7248, $p=0.00786$ vs. 6217.1 ± 495 wheel rotations per day).

The ABA model combines voluntary physical activity in a running wheel and time-restricted feeding to reduce body weight. Our data suggest the usefulness of the model to explain pathophysiological alterations occurring in AN, with special regard to the role of biogenic amines in the enteric nervous system.

BENEFITS AND HEALTH RISKS OF FOOD SUPPLEMENTS CONSUMPTION

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Food supplements are the type of food that is defined as concentrated sources of nutrients (i.e. mineral and vitamins) or other substances with a nutritional or physiological effect that is marketed in "dose" form (e.g. pills, tablets, capsules, liquids in measured doses). This type of food may contain different types of components including vitamins, minerals, amino acids, essential fatty acids, fibre and various plants and herbal extracts. Over the last years, food supplements market has increased significantly. The number of new products on the market is increasing. In 2017, the Chief Sanitary Inspector was informed about the 12556 new products on the market while in 2011 it was only 1969 products.

The Chief Sanitary Inspector monitors the supplement market in Poland. In 2017, 1032 investigations were carried out following which 6.24% of samples were disqualified. Chief Sanitary Inspector notified about several substances that are not allowed in food. The most dangerous discoveries were the detection of yohimbine, vinpocetine, 5-HTP, isopropylennyrine, N-methyltimine, evodiamine, phenylethylamine, hordenine, pikamilon, sildenafil, agmatine in food supplements.

On the food market, there are more and more products containing substances that can not be found in food, such as dangerous substances without a history of consumption, or doping. Thanks to the information exchange systems functioning in the European Union, it is possible to quickly exchange information about the statement on the market of dangerous products. A key tool to ensure the flow of information to enabling swift reaction when risks to public health are detected in the food chain is RASFF – the Rapid Alert System for Food and Feed.

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