XV Conference
XV Konferencja

23-25 October 2014
Lodz, Poland

Biogenic Amines and Related Biologically Active Compounds

Aminy biogenne i pokrewne związki o wysokiej aktywności biologicznej

Invited Speakers:

Nicholas Carruthers (USA), Madeleine Ennis (UK), Pertti Panula (Finland), Maria Beatrice Passani (Italy), Holger Stark (Germany), Satoshi Tanaka (Japan)

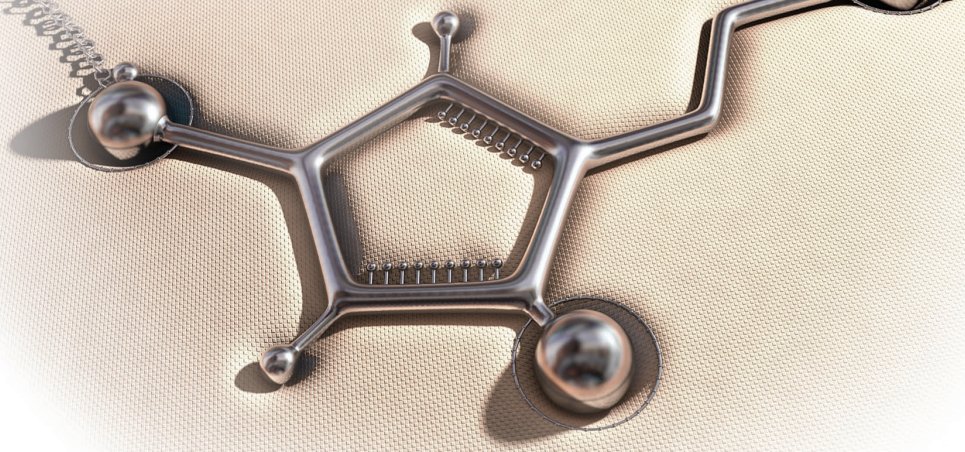
Dear Colleagues,

We have again an even year (2014) and a new, although it is the 25th, Conference of our Society devoted to biogenic amines, small and rather simple chemicals which nevertheless have a great impact on the functioning of all living organisms.

For about a century, scientists around the whole globe have been trying to uncover the mystery of the action of these molecules, and each time, along with some successful insight into the World of Biogenic Amines, a new, more complex interplay among the constituents of the system is revealed.

I believe the invited speakers, who are extremely knowledgeable in the field, will familiarize us with the issue and bring about better understanding of these complicated relations at chemical, biochemical, molecular biology and clinical levels.

We are all here to share expertise and experience and also a fresh look.
I welcome you heartily to the meeting and to the charm of Lodz in your free time,



W. Agnieszka Fogel

The President

CONFERENCE PROGRAMME

Hotel Ambasador Centrum, Lodz, Poland

Thursday, October 23rd 2014

14.00	Arrival, accommodation Ambasador Centrum Hotel, Piłsudskiego 29 St., 90-307 Lodz
16.00 – 19.00	Registration: Ambasador Centrum Hotel, Reception Hall
17.00 – 19.00	Poster mounting, Conference room B
19.00	**Opening Ceremony:** 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* **Conference Lecture:** **DISCOVERY AND CHARACTERIZATION OF SELECTIVE OREXIN RECEPTOR ANTAGONISTS** Nicholas I. Carruthers *Janssen Pharmaceutical Research & Development, L.L.C., San Diego, USA*
19.00 – 22.00	Welcome Reception Ambasador Centrum Hotel, Restaurant

Friday, October 24th 2014

9.00 – 10.50	**Session I,** **chaired by: W. Agnieszka Fogel and Jerzy Jochem**
9.00 – 9.30 Invited Lecture	**THE MANY BEHAVIOURAL FACETS OF BRAIN HISTAMINE** M. Beatrice Passani *Department of Neuroscience, Psychology, Drug Development and Child Care, University of Firenze, Firenze, Italy*
9.30 – 9.45 O1	**THE CENTRAL HISTAMINERGIC SYSTEM PARTICIPATES IN NEUROPEPTIDE Y RECEPTOR-EVOKED CARDIOVASCULAR EFFECTS IN HAEMORRHAGE-SHOCKED RATS** Adam Krawiec, Karolina Jasikowska, Jerzy Jochem *Department of Basic Medical Sciences, Medical University of Silesia, Bytom, Poland*
9.45 – 10.15 Invited Lecture	**INTERACTIONS OF THE HISTAMINERGIC AND DOPAMINERGIC SYSTEMS IN THE BRAIN: IMPLICATIONS IN ALCOHOL-RELATED BEHAVIOR AND TOURETTE SYNDROME** Pertti Panula *Neuroscience Center and Institute of Biomedicine, University of Helsinki, Helsinki, Finland*
10.15 – 10.20 P1	**SALSOLINOL INDUCED NEUROINFLAMMATION** Magdalena Kurnik, Krzysztof Gil, Andrzej Bugajski, Piotr Thor *Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland*
10.20 – 10.50	*Coffee break and poster viewing*
10.50 – 12.00	**Session II,** **chaired by: Katarzyna Kieć-Kononowicz and Dariusz Matosiuk**
10.50 – 11.20 Invited Lecture	**THIAZOLE DERIVATIVES AS DOPAMINE D_3 RECEPTOR AGONISTS WITH HIGH SELECTIVITY AND IN VIVO ACTIVITY** Holger Stark, Tim Kottke, Eva M. Eichelsbacher, Neda Bakthiari, Jukka M. Leppanen, Britta C. Sasse, Oliver Saur, Michael P. Hill, Alan Crossman, Ervan Bezard *Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Duesseldorf, Duesseldorf, Germany; Institute of Pharmaceutical Chemistry, Goethe University, Biozentrum, Frankfurt am Main, Germany; Department of Pharmaceutical Chemistry, University Kuopio, Kuopio, Finland; Motac Neuroscience Ltd., Manchester, United Kingdom; Lab. Neurophysiologie CNRS UMR 5543, Bordeaux, France*
11.20 – 11.35 O2	**NON-IMIDAZOLE HISTAMINE H_3 LIGANDS. SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVSTIGATION THIAZOLE-TYPE ANTAGONISTS LACKING A BASIC SIDE CHAIN** Marek Staszewski, Roman Guryn, Piotr Kopczacki, Krzysztof Walczyński *Department of Synthesis and Technology of Drugs, Medical University of Lodz, Poland; Polfarmex S.A., Kutno, Poland*

11.35 – 11.40
P2

ACETYL- AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY OF CHLOROPHENOXY DERIVATIVES - HISTAMINE H_3 RECEPTOR LIGANDS
Dorota Łażewska, Anna Więckowska, Natalia Guzior, Caittin Moore, Kamil Kuder, Barbara Malawska, Katarzyna Kieć-Kononowicz
Department of Technology and Biotechnology of Drugs & Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Cracow, Poland

11.40 – 11.45
P3

THE EFFECT OF A COMBINATION OF HISTAMINE H_3 RECEPTOR ANTAGONISTS/INVERSE AGONISTS AND DONEPEZIL IN MICE PASSIVE AVOIDANCE TEST
Szczepan Mogilski, Monika Kubacka Dorota Łażewska, Barbara Filipek, Katarzyna Kieć-Kononowicz
Department of Pharmacodynamic, Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Cracow, Poland

11.45 – 11.50
P4

THE CHARACTERIZATION OF SELECTED ADME-TOX PARAMETERS OF NEW POTENT HISTAMINE H_3R LIGANDS
Gniewomir Latacz, Michał Malahfji, Katarzyna Kieć-Kononowicz
Collegium Medicum Jagiellonian University, Department of Technology and Biotechnology of Drugs, Cracow, Poland

11.50 – 11.55
P5

HISTAMINE INTERACTION WITH HISTAMINE H_4 RECEPTOR – A STUDY OF BIOGENIC AMINE MODULATORY EFFECT ON HUMAN EOSINOPHILS FUNCTION
Marek Grosicki, Katarzyna Kieć-Kononowicz
Jagiellonian University Medical College, Faculty of Pharmacy, Department of Technology and Biotechnology of Drugs, Cracow, Poland

11.55 – 12.00
P6

H_4R ANTAGONIST AND OPIOID RECEPTOR ANTAGONISTS INTERACTIONS IN ANTINOCICEPTION
Przemysław Rzodkiewicz, Emilia Gąsińska, Dorota Łażewska, Katarzyna Kieć-Kononowicz, Dariusz Szukiewicz, Magdalena Bujalska-Zadrożyny, Sławomir Maśliński
Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland; Department of Pharmacodynamics, CEPT Laboratory, Medical University of Warsaw, Poland; Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Department of Ge-neral and Experimental Pathology, Medical University of Warsaw, Poland

12.30 – 14.00 Lunch

14.00 – 15.10 **Session III,**
chaired by: Anna Stasiak and Krzysztof Walczyński

14.00 – 14.30
Invited Lecture

MAST CELLS – NOT JUST IMPORTANT IN ALLERGY!
Madeleine Ennis
Centre for Infection and Immunity, The Queen's University of Belfast, UK

14.30 – 14.45
O3

TLR3- AND TLR7-MEDIATED PHENOTYPE ALTERATIONS AND INTERFERON TYPE I SYNTHESIS AS AN EVIDENCE FOR MAST CELL ROLE IN VIRAL INFECTIONS
Piotr Witczak, Aleksandra Słodka, Ewa Brzezińska-Błaszczyk
Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

14.45 – 14.50
P7

CYSTEINYL LEUKOTRIENE RECEPTOR (CYSTLTR1, CYSLTR2, GPR17) EXPRESSION ON MATURE TISSUE MAST CELLS
Karolina Wódz, Magdalena Efenberger, Ewa Brzezińska-Błaszczyk
Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

14.50 – 15.05
O4

PLACENTAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PLACENTA GROWTH FACTOR (PIGF) RECEPTORS IN RELATION TO MATERNAL HEMOGLOBIN CONCENTRATION AND PREGNANCY OUTCOME
Aleksandra Stangret, Ilona Dudek, Marta Skoda, Dariusz Szukiewicz
Department of General & Experimental Pathology with Centre for Preclinical Research and Technology (CEPT), Medical University of Warsaw, Warsaw, Poland

15.05 – 15.10
P8

EXPRESSION PROFILE OF KININ-RELATED GENES IN HUMAN RENAL PROXIMAL TUBULE CELLS IN RESPONSE TO AMPHOTERICIN B TREATMENT
Małgorzata Kimsa, Joanna Gola, Celina Kruszniewska-Rajs, Mariusz Gagoś, Grzegorz Czernel, Urszula Mazurek, Barbara Strzałka-Mrozik
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; Univesity of Life Sciences in Lublin, Department of Biophysics, Lublin, Poland; Institute of Biology and Biotechnology, Maria Curie-Skłodowska University, Department of Cell Biology, Lublin, Poland

15.10 – 15.30 Coffee break and poster viewing

15.30 – 16.30 General Assembly of the Polish Histamine Research Society

16.45 Sandwich /Coffee /Tea

17.50 Bus transfer to the Musical Theatre in Lodz

18.30 Cabaret "Not time to sleep yet", the Musical Theatre in Lodz
Cast: Rafał Kmita Cabaret Group, the Soloists, Choir, the Ballet and Orchestra of the Musical Theatre in Lodz

Transfer back

21.30 Dinner
Ambasador Centrum Hotel, Restaurant

Saturday, October 25th 2014

Session IV,
chaired by: Barbara Skrzydło-Radomańska and Dariusz Szukiewicz

9.30 – 10.00 Invited Lecture

INDUCTION OF HISTAMINE SYNTHESIS IN CD11b^{+} Gr-1^{+} MYELOCYTES AND ITS IMMUNOLOGICAL ROLES IN A MURINE SYNGENEIC TUMOR MODEL
Satoshi Tanaka
Department of Immunobiology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University

10.00 – 10.15 O5

RECEPTOR AND NON-RECEPTOR MEDIATED BIOLOGICAL EFFECTS OF L- AND D-LACTATE ON CANCEROUS CELLS IN VITRO.
Waldemar Wagner, Wojciech Ciszewski, Katarzyna Kania, Jarosław Dastych
Laboratory of Cellular Immunology, Institute for Medical Biology Polish Academy of Science, Lodz, Poland; Laboratory of Transcriptional Regulation, Institute for Medical Biology Polish Academy of Science, Lodz, Poland

10.15 – 10.30 O6

ANGIOGENIC ACTIVITY OF ASCITIC COMPOUNDS IN THE COURSE OF OVARIAN CANCER
Krzysztof Gawrychowski, Grzegorz Szewczyk, Ewa Skopińska-Różewska, Michał Pyzlak, Ewa Barcz, Dariusz Szukiewicz, Piotr Skopiński
Department of Gynecological Oncology and Oncology, Medicover Hospital, Warsaw, Poland; Department of Obstetrics and Gynecology, Institute of Mother and Child, Warsaw, Poland; Chair and Department of General and Experimental Pathology, Warsaw Medical University, Warsaw, Poland; Department of Pathology, Centre of Biostructure Research, Warsaw Medical University, Warsaw, Poland; Ist Department of Obstetrics and Gynecology, Warsaw Medical University, Warsaw, Poland; Department of Histology and Embryology, Centre of Biostructure Research, Warsaw Medical University, Warsaw, Poland

10.30 – 10.35 P9

TRANSCRIPTION ACTIVITY OF MELATONIN-RELATED GENES IN ENDOMETRIAL CANCER
Agnieszka Jęda, Andrzej Witek, Joanna Orchel, Aleksandra Skubis, Bartosz Sikora, Justyna Szota-Czyż, Tomasz Janikowski, Michał Baliś, Urszula Mazurek
Department and Clinic of Gynecology and Obstetrics, Medical University of Silesia, Katowice, Poland; Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland

10.35 – 10.40 P10

SUPPRESSOR GENE EXPRESSION PROFILE IN RPTEC CELLS TREATED WITH AMPHOTERICIN B
Aleksandra Skubis, Joanna Gola, Celina Kruszniewska-Rajs, Bartosz Sikora, Małgorzata Kimsa, Mariusz Gagoś, Grzegorz Czernel, Urszula Mazurek, Barbara Strzałka-Mrozik
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; University of Life Sciences in Lublin, Department of Biophysics, Lublin, Poland; Institute of Biology and Biotechnology, Maria Curie-Skłodowska University, Department of Cell Biology, Lublin, Poland

10.40 – 10.45 P11

INFLUENCE OF AMPHOTERICIN B ON HISTAMINE RELATED GENES IN RPTEC CELLS
Celina Kruszniewska-Rajs, Bartłomiej Skowronek, Joanna Gola, Małgorzata Kimsa, Adrian Janiszewski, Mariusz Gagoś, Grzegorz Czernel, Urszula Mazurek, Barbara Strzałka-Mrozik
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; University of Life Sciences in Lublin, Department of Biophysics, Lublin, Poland; Institute of Biology and Biotechnology, Maria Curie-Skłodowska University, Department of Cell Biology, Lublin, Poland

10.45 – 10.50 P12

PROFILE OF MELATONIN RELATED GENES EXPRESSION IN RPTEC CELLS TREATED WITH AMPHOTERICIN B
Bartosz Sikora, Joanna Gola, Celina Kruszniewska-Rajs, Aleksandra Skubis, Małgorzata Kimsa, Mariusz Gagoś, Grzegorz Czernel, Urszula Mazurek, Barbara Strzałka-Mrozik
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; University of Life Sciences in Lublin, Department of Biophysics, Lublin, Poland; [3]Institute of Biology and Biotechnology, Maria Curie-Skłodowska University, Department of Cell Biology, Lublin, Poland

10.50 – 10.55 P13

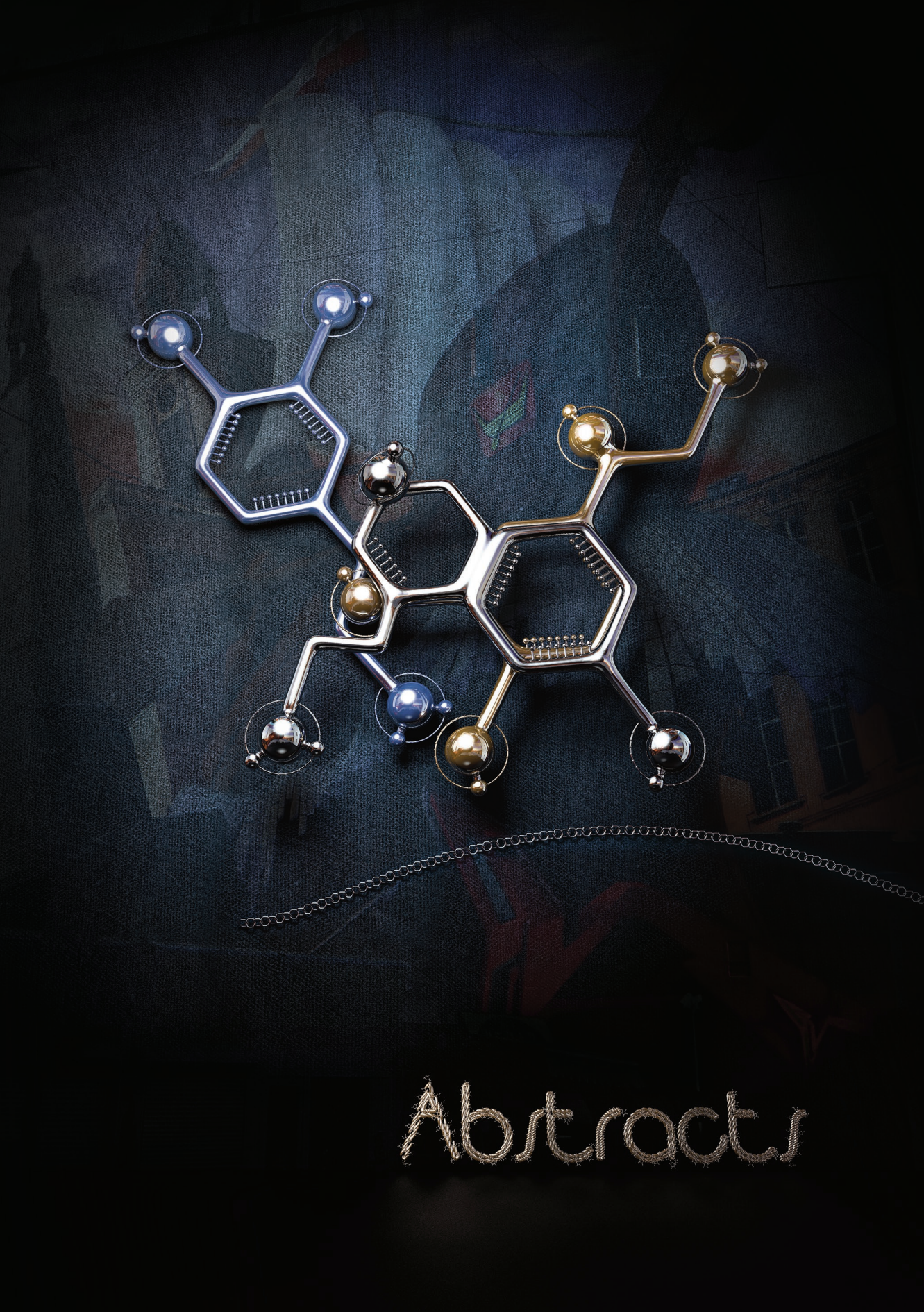
INDUCTION OF TNF AND SEROTONIN PATHWAYS IN RPTEC CELLS TREATED WITH AMPHOTERICIN B
Joanna Gola, Adrian Janiszewski, Celina Kruszniewska-Rajs, Małgorzata Kimsa, Bartłomiej Skowronek, Mariusz Gagoś, Grzegorz Czernel, Urszula Mazurek, Barbara Strzałka-Mrozik
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; University of Life Sciences in Lublin, Department of Biophysics, Lublin, Poland; Institute of Biology and Biotechnology, Maria Curie-Skłodowska University, Department of Cell Biology, Lublin, Poland

10.55 Closing Ceremony of the XV-th Conference of the Polish Histamine Research Society
Coffee /Tea

12.00 Lunch

18.30 Opera: Gioacchino Rossini “Il Barbiere di Siviglia” (performance of the students of the Academy of Music in Lodz), the Grand Theater Lodz
Cast: The Choir, the Ballet and Orchestra of the Grand Theater Lodz
Conductor: Eraldo Salmieri

21.30 Dinner
Ambasador Centrum Hotel, Restaurant


Abstracts

DISCOVERY AND CHARACTERIZATION OF SELECTIVE OREXIN RECEPTOR ANTAGONISTS

Nicholas I. Carruthers

Janssen Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Row, San Diego, CA, 92121, USA

Invited Lecture

In 1998, De Lecea and Sakurai independently reported the existence of the orexin neuropeptides hypocretin-1 (hcrt-1) and hypocretin-2 (hcrt-2) which were also termed orexin-A and orexin-B. These neuropeptides were shown to originate in the hypothalamus from a common precursor and project widely to key areas of the central nervous system (CNS) hypothesized to control sleep-wake states, modulation of food intake, panic, anxiety, reward and addictive behaviors. The orexin neuropeptides mediate their effect by stimulating two distinct G-protein coupled receptors, orexin-1 (OX1) and orexin-2 (OX2). These receptors are co-located or selectively located in certain areas of the CNS suggesting differentiated roles. For example, OX1Rs are selectively expressed in the bed nucleus of the stria terminalis, amygdala, cingulate cortex and locus coeruleus which play a role in panic and anxiety. Conversely, OX2Rs are exclusively expressed in histaminergic neurons in the tuberomammillary nuclei which play a critical role in wake promotion. Interest in the orexin system has led to at least four dual orexin receptor antagonists entering human trials for the treatment of sleep related disorders. We have shown pre-clinically that selective antagonism of the OX2R is sufficient to initiate and prolong sleep in rodents. Selectively targeting the OX1R and its role in more complex emotional behavior (panic, anxiety) is now emerging. For example, sodium lactate infusion or acute hypercapnia, which causes panic in humans and are used as an animal model of panic, activates orexin neurons in the perifornical hypothalamus. This activation correlates with anxiety in the social interaction test or open field test. Blocking the activation with either siRNA or selective OX1 receptor antagonists attenuates these panic-like responses. In order to further validate the role of the OX1 receptor, characterization of improved tool compounds in animal models is required. Presented here will be the discovery, synthetic methods and SAR associated with novel selective orexin receptor antagonists.

THE MANY BEHAVIOURAL FACETS OF BRAIN HISTAMINE

M. Beatrice Passani

Department of Neuroscience, Psychology, Drug Development and Child Care, University of Firenze, Viale Pieraccini 6, 50139 Firenze, Italy

Invited Lecture

The last few decades have witnessed an increasing interest for, and knowledge of the neurobiology of histamine. An evergrowing number of publications demonstrate the physiological role of brain histamine in the regulation of homeostatic functions and behaviours such as wakefulness, appetite, memory consolidation and extinction. Dysregulation of histamine synthesis or neurotransmission has been associated with human brain disorders, whereas histamine receptor ligands have been proposed for the treatment of CNS diseases.

In our laboratory, we have extensively studied in animal models the involvement of the histaminergic system in learning and storing aversive, as well as emotionally neutral information. By using behavioral tests such as contextual fear conditioning, inhibitory avoidance and object recognition in association with systemic or intracerebral administration of selective histaminergic ligands, and in-vivo microdialysis, we described how activation of different subpopulation of histaminergic receptors (H_1, H_2 and H_3) in selected brain regions exerts promnesic or amnesic effects.

We also demonstrated in animal models, that the efficacy of compounds commonly used in the treatment of human brain disorders require the integrity of the histaminergic system to exert their behavioral and neurochemical effects.

More recently, we focused our attention on brain histamine as a neurotransmitter regulating feeding behaviour. Hunger and satiety are key factors driving eating behaviour. They are controlled by a complex interplay of central neurotransmitter systems and peripheral endocrine stimuli. Brain histamine is released during the appetitive phase to provide a high level of arousal preparatory to feeding and mediates satiety. The lipid-derived messenger oleoylethanolamide (OEA) is released by enterocytes in response to fat intake and indirectly signals satiety to hypothalamic nuclei. However, despite the possible functional overlap of satiety signals it is not known whether histamine participates in OEA-induced hypophagia. Using different behavioural, neurochemical and neuroanatomical approaches, we reported that OEA requires the integrity of the brain histamine system to fully exert its hypophagic effect. We also described the oxytocin rich neurons in the paraventricula nucleus as the likely hypothalamic area where brain histamine influences the central effects of OEA.

In conclusion, we believe that our studies may contribute to the development of more effective pharmacotherapy for the management of cognitive and eating disorders and ameliorate the safety profile of centrally acting drugs.

THE CENTRAL HISTAMINERGIC SYSTEM PARTICIPATES IN NEUROPEPTIDE Y RECEPTOR-EVOKED CARDIOVASCULAR EFFECTS IN HAEMORRHAGE-SHOCKED RATS

Adam Krawiec, Karolina Jasikowska, Jerzy Jochem

Department of Basic Medical Sciences, Medical University of Silesia, Katowice, Piekarska 18, 41-902 Bytom, Poland

The central histaminergic system is able to induce the reversal of experimental haemorrhagic hypotension due to the activation of the sympathetic and renin-angiotensin systems as well as secretion of arginine vasopressin and proopiomelanocortin-derived peptides. On the other hand, our preliminary data demonstrate a depressor effect resulting from intracerebroventricular (icv) administration of neuropeptide Y (NPY) in haemorrhage-shocked rats. Since histaminergic neurones of the tuberomammillary nucleus receive input from neurones producing NPY localized in the caudal magnocellular nucleus of the hypothalamus, the aim of the present study was to examine (1) cardiovascular effects of NPY receptor antagonists in haemorrhagic shock and (2) a possible involvement of the histaminergic system in their action. Experiments were performed in ketamine/xylazine (100 mg/kg + 10 mg/kg, intraperitoneally)-anaesthetised male Wistar rats subjected for an irreversible haemorrhagic shock produced by intermittent blood withdrawal over a period of 15-25 min, until mean arterial pressure (MAP; TAM-A transducer amplifier module, Hugo Sachs Elektronik, Germany) decreased to and stabilised at 20-25 mmHg, which was associated with the death of all animals within 30-40 min in the control group. NPY receptor type 1 and 2 antagonists – BIBP 3226 (25 nmol, icv) and BIIE 0246 (1 μmol, icv), respectively, induced long-lasting rises in MAP and renal blood flow (RBF; TTFM transit time flowmeter module, Transonic Systems Inc., USA) as well as the increase in survival rate of 2 h up to 100%, with no influence on heart rate (HR; ECGA amplifier, Hugo Sachs Elektronik, Germany). These effects were completely blocked by pre-treatment with prazosin (0.5 mg/kg, intravenously) and partially inhibited by pre-treatment with chlorpheniramine (50 nmol, icv), and thioperamide (50 nmol, icv), but not with ranitidine (50 nmol, icv). In the control, previously described groups, prazosin and histamine receptor antagonists given alone did not evoke long-lasting changes in measured parameters and did not influence the survival rate at 2 h in the used model of haemorrhagic shock. In conclusion, (1) centrally acting NPY receptor type 1 and 2 antagonists evoke a long-lasting pressor effect in haemorrhage-shocked rats due to the activation of the sympathetic system, and (2) the central histaminergic system is involved in NPY receptor antagonists-induced cardiovascular action.

INTERACTIONS OF THE HISTAMINERGIC AND DOPAMINERGIC SYSTEMS IN THE BRAIN: IMPLICATIONS IN ALCOHOL-RELATED BEHAVIOR AND TOURETTE SYNDROME

Pertti Panula

Neuroscience Center and Institute of Biomedicine, University of Helsinki, Helsinki, Finland

Invited Lecture

Recent findings have suggested a role for the brain histaminergic system in alcohol-related behaviors, sensorimotor gating and in Tourette syndrome. Animal model studies have implicated that histamine H_3 receptor (H_3R) regulates alcohol reward and consumption, possibly via mechanism involving an interaction with the brain dopaminergic system. We studied the role of H_3Rs in alcohol-related behaviors using H_3R knockout (KO) mice and ligands. H_3R KO mice consumed less alcohol than wildtype (WT) mice in a two-bottle free choice test and in a "Drinking in the dark" -model. H_3R antagonist ciproxifan suppressed and H_3R agonist immepip increased alcohol drinking in C57BL/6J mice. Impairment in reward mechanisms in H_3R KO mice was confirmed by the lack of alcohol-evoked conditioned place preference.

We also studied sensorimotor gating in histidine decarboxylase (HDC KO), H_1 receptor (H_1R KO) and H_3 receptor (H_3R KO) knockout mice using the prepulse inhibition (PPI) method. The locomotor activation by dopaminergic drugs was also assessed. The dopaminergic functions were investigated by radioactive in situ hybridization and by semi-quantitative Western blotting.

We found that H_3R KO but not HDC KO or H_1R KO mice have impaired PPI indicating a deficiency in sensorimotor gating. Impaired PPI in H_3R KO mice was accompanied with altered striatal dopaminergic signal transduction, indicated by the lack of striatal extracellular signal-regulated kinase 1/2 (ERK1/2) activation in response to systemic administration of dopamine D_1 and D_2 receptor agonists (SKF-38393 and quinpirole, respectively). H_3R KO mice also displayed lower levels of D_1 receptor mRNA in the striatum compared to control mice.

Taken together, these findings demonstrate that H_3R is an important regulator of sensorimotor gating and the mechanism by which H_3R regulates this involves interaction with the striatal dopaminergic system. The results are import for understanding the role of histamine and H_3R in Tourette syndrome and alcohol dependence.

SALSOLINOL INDUCED NEUROINFLAMMATION

Magdalena Kurnik, Krzysztof Gil, Andrzej Bugajski, Piotr Thor

Department of Pathophysiology, Jagiellonian University Medical College, Krakow, Poland

Aim. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, SAL) is an endogenous compound thought to be involved in the etiology of Parkinson's disease (PD). Neuroinflammation is thought to be a major contributor to the neuronal degeneration in PD. The alteration of inflammatory cytokines in the brain, cerebral spinal fluid and plasma of PD patients supports the existence of functional connections between the immune and nervous systems. In animal studies, chronic administration of SAL induced parkinsonian-like symptoms. However, little has been known about the effects of SAL on the cytokine production or hypothalamo-pituitary axis (HPA) activation.

Materials and methods. The aim of the study was to evaluate the influence of exogenous SAL on serum levels of corticosterone, CRF and IL-1β. Wistar rats were subjected to intraperitoneal dosing of SAL (200 mg/kg) with osmotic mini-pumps for 2 (S1, n=8) or 4 weeks (S2, n=8). An equivalent group of rats served as the control (C). At the end of the experiment blood samples were collected and assayed by ELISA according to the manufacturer's instruction.

Results. The serum levels of CRF, corticosterone and IL-1β were elevated in both salsolinol-treated groups in comparison with the C group (S1=0.0944 ng/ml ± 0.062, S2=0.0916 ng/ml ± 0.036, C=0.0708 ng/ml ± 0.025; S1=34.29 ng/ml ± 3.7, S2=28.24 ng/ml ± 6.2, C=16.94 ng/ml ± 9.9; S1=455.68 pg/ml ± 134.6, S2=491.62 pg/ml ± 120.4, C=321.79 pg/ml ± 122.6, respectively). No differences between S1 and S2 groups were observed.

Conclusion. Peripheral SAL administration evokes changes in HPA activity. Our previous results showed that SAL causes mast cells (MC) degranulation in the gut. Once activated, MC may secrete a range of neurosensitizing and proinflammatory molecules, increasing gut-blood and blood-brain barrier permeability. For example, IL-1β is involved in the activation of the HPA, by stimulating hypothalamic CRF secretion and/or by activation of an intra-adrenal CRF/ACTH system. These results serve as an additional support for the existence of a relationship between the nervous, neuroendocrine and immune systems.

THIAZOLE DERIVATIVES AS DOPAMINE D_3 RECEPTOR AGONISTS WITH HIGH SELECTIVITY AND IN VIVO ACTIVITY

Holger Stark[1], Tim Kottke[2], Eva M. Eichelsbacher[2], Neda Bakthiari[2], Jukka M. Leppanen[2,3], Britta C. Sasse[2], Oliver Saur[2], Michael P. Hill[4], Alan Crossman[4], Ervan Bezard[4,5]

[1]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Duesseldorf, D-40225 Duesseldorf, Germany;
[2]Institute of Pharmaceutical Chemistry, Goethe University, Biozentrum, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany;
[3]Department of Pharmaceutical Chemistry, University Kuopio, Kuopio, Finland;
[4]Motac Neuroscience Ltd., Manchester, United Kingdom;
[5]Lab. Neurophysiologie CNRS UMR 5543, Bordeaux, France

Invited Lecture

L-DOPA is still the gold standard treatment for motor functions with dopamine substitution therapy in patients with Parkinson´s disease. Dopamine receptor subtype agonists have great influence on therapeutic options as an ideal dopamine receptor agonist should fulfill the following criteria: 1) a physiological receptor profile with good antiparkinson efficacy, 2) a good brain distribution, 3) oral bioavailability, 4) rapid onset, 5) long acting and 6) no unwanted side-effects. Although a small number of non-ergot derivatives are on the market, no single drug available fulfills all the criteria.

In a long-termed development program we have changed the 2-aminothiazole motif of pramipexole as a prototypical catechol bioisosteric moiety by removing the aromatic amino functionality as described previously with etrabamine [1,2]. This derivatisation maintained or improved affinity at dopamine D_3 receptor subtype, maintained agonist properties and simulated binding profile at dopamine D_2-like receptor family. Depending on the substitution pattern on the core pharmacophore element a series of highly affine and selective agonists have been developed. Selected compounds were screened on unilateral 6-OHDA-lesioned rat model of Parkinsons´s disease and further selection on MTPP-treated marmoset model for their antiparkinsonian efficacy in comparison to L-DOPA, apomorphine and ropinerole. At least two compounds simultaneously fulfilled all the criteria mentioned above and showed high drug potential due to the results of the initial preclinical toxicological screenings. Studies on functional signaling based on [^{35}S]GTPγS shift and on $ERK_{1/2}$ phosphorylation showed significant differences and a good predictive factor for in vivo activities.

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Support by the Alexander-von-Humboldt foundation, the EU COST Actions CM1103 and 1207 as well as the DFG INST 208/664-1 FUGG is greatly acknowledged.

NON-IMIDAZOLE HISTAMINE H_3 LIGANDS. SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVESTIGATION THIAZOLE-TYPE ANTAGONISTS LACKING A BASIC SIDE CHAIN

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Aim. The aim of this study was the synthesis and preliminary pharmacological investigation of new 1-[2-thiazol-4-yl-(4-subsitutedphenyl)]-4-*n*-propylpiperazines, 1-[2-thiazol-5-yl-(4-subsitutedphenyl)]-4-*n*-propylpierazines and 1-[2-thiazol-5-yl-(ω-phenylalkyl)]-4-*n*-propylpiperazines as H_3 histamine receptor antagonists.

Previously described series of non-imidazole piperazine-based histamine H_3 antagonists, consisting of 1-[2-thiazol-5-yl-(2-aminoethyl)]- [1] and 1-[2-thiazol-4-yl-(2-aminoethyl)]- moieties [2] showed moderate to pronounced affinity for the receptor and lead to potent and selective H_3 antagonist – 1-[2-Thiazol-5-yl-(2-(*N*-methyl-*N*-phenylpropyl)aminoethyl)]4-n-propylpiperazine. Following this discovery we sought to explore replacements for the *N,N*-disubstitutedaminoethyl chain by phenyl or phenylalkyl substituent at position 4 or 5 of thiazole ring to compare influence on H_3-receptor antagonistic activity.

Materials and methods. Synthesized compounds were tested *in vitro*, using the electrically stimulated to contraction guinea pig jejunum [3] – H_3 receptor antagonists or additionally histamine inducted to contraction guinea pig jejunum – H_1 receptor antagonists [4].

Results and conclusion. Throughout the study, we chose to retain 2-thiazol-4-n-propylpiperazine fragment which is optimal according to our previous results. We turned out our attention to removing nitrogen nucleus of the side chain by replacing *N,N*-disubstitutedaminoethyl residue by phenyl moiety to afford 1-[2-thiazol-4-yl-(4-subsitutedphenyl)]-4-n-propylpiperazines and 1-[2-thiazol-5-yl-(4-subsitutedphenyl)]-4-n-propylpiperazines. The highest potency for both homologous series was seen for compound carrying on unsubstituted benzene moiety at position 5 of thiazole ring. These results prompted us to replace benzene ring by series of phenylalkyl chain at position 5 of 1-(2-thiazol-5-yl)-4-*n*-propylpiperazine moiety. These results suggest that, side chain in the 2-thiazol-4-n-propylpiperazine scaffold should contain a basic center and should be present at a favourable position 5 of thiazole ring.

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ACETYL- AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY OF CHLOROPHENOXY DERIVATIVES - HISTAMINE H_3 RECEPTOR LIGANDS

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Aim. Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder and the most common form of dementia. The currently 5 drugs are approved for the symptomatic treatment of AD and there is an urgent need to find new therapies for AD [1]. One of possibilities are multi-acting anti-AD ligands [2]. Histamine H_3R antagonists/inverse agonists have been shown to exert pro-cognitive effects in pre-clinical models and some of them are/were tested in clinical trials, e.g. ABT-288, GSK239512, MK-0249 [3]. Combination of cholinesterases (AChE/BuChE) inhibiting and histamine H_3 receptor (H_3R) antagonizing properties in a single molecule might show synergistic effects to improve cognitive deficits in AD. The aim of this study was to evaluate AChE and BuChE inhibitory activity of some histamine H_3R ligands synthesized by our research group [4].

Materials and methods. AChE and BuChE inhibitory activity was evaluated by spectrophotometrical Ellamn's method using AChE from electric eel and BuChE from horse serum (2.5 units/1 mL) [5].

Results. Most of the tested compounds displayed moderate inhibitory activity for both cholinesterases (AChE and BuChE), ranging IC_{50} from 0.90 μM to 18.57 μM. Compounds are not selective inhibitors of both cholinesterases but IC_{50} values against BuChE are slightly lower. The obtained results were compared with moderate histamine H_3 receptor affinity of tested compounds (hH$_3$R K_i = 128-805 nM) [4]. Among investigated structures with different amine moieties (piperidine, 3-methylpiperidine, 4-methylpiperidine and homopiperidine) the highest inhibitory activity showed homopiperidine derivatives.

Conclusions. Homopiperidine derivatives turned out to be the most active against AChE and BuChE. They may serve as a starting point in the further discovery of dual-acting compounds cholinesterases inhibitors and anti-H_3 ligands.

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This project was financed by a grant from the National Science Center based on decision No DEC-2011/02/A/NZ4/00031 and by the JU CM grant No K/DSC/000082.

THE EFFECT OF A COMBINATION OF HISTAMINE H_3 RECEPTOR ANTAGONISTS/INVERSE AGONISTS AND DONEPEZIL IN MICE PASSIVE AVOIDANCE TEST

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Aim. Impaired acetylcholine (Ach) neurotransmission is associated with deficits of cognitive-related functioning. Histamine H_3 receptors (H_3Rs), which are distributed throughout the brain, regulate the activity of different neurotransmitters including acetylcholine (ACh). The goal of these studies was to evaluate the behavioral effects of Pitolisant and DL-76, a selective non-imidazole histamine H_3Rs antagonists/inverse agonists, in mice model of memory impairment.

Materials and methods. The effects of investigated compounds on histamine H_3Rs agonist-induced water intake in male rats were tested (dipsogenia model induced by R-α-methylhistamine, RAMH) in order to confirm their antagonistic properties for H_3Rs. Moreover, the ability of acute treatment of Donepezil, Pitolisant and DL-76 to influence memory acquisition and consolidation in mice was investigated in a passive avoidance paradigm in which scopolamine was used to induce a cholinergic deficit and subsequent memory and learning impairment. Finally, a combination of lower doses of H_3Rs antagonists with donepezil was investigated.

Results. The antagonism of the histamine H_3Rs agonist *RAMH*-induced drinking response in the rat dipsogenia model was demonstrated for both Pitolisant and DL-76. Furthermore, the significant improvement of scopolamine-induced memory deficits was observed in the Passive Avoidance Paradigm in mice for histamine H_3Rs antagonists as well as for acetylcholine esterase inhibitor (Donepezil). Concomitant administration of histamine H_3Rs antagonists with Donepezil was associated with higher efficacy.

Conclusions. These results confirm that blockade of histamine H_3Rs might have therapeutic utility for the treatment of memory deficits and learning disorders. Moreover, it has been demonstrated that the combination of two different and independent mechanisms leading to increased Ach neurotransmission may result in higher efficacy of the treatment of memory deficits and can lead to reduced drug dosage, which may be associated with better tolerance of the therapy.

Supported by National Science Center DEC-2011/02/A/NZ4/00031.

THE CHARACTERIZATION OF SELECTED ADME-TOX PARAMETERS OF NEW POTENT HISTAMINE H_3R LIGANDS

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Aim. The compounds which have acceptable ADME-TOX parameters are defined as a drug-like and are an ideal candidates to the human Phase I clinical trials. To the advantages of the *in vitro* and *in silico* assays, which allow to estimate drug-likeness of new molecular entities (NMEs) at an early stage of drug development, belong time and funds saving, high-throughput screening and elimination of animals [1]. In this work we determined two very important ADME-TOX parameters – toxicity and metabolic stability of the series of new ligands with very high histamine H_3 receptor affinity (hH_3R K_i = 8.8 – 23.4 nM).

Materials and methods. *In silico* MetaSite computational method was used to specify the routes of metabolic biotransformation of H_3R ligands and to predict the most probably structural formulas of the metabolites [2]. The results were next compared with the LC-MS spectra of the products of biotransformation *in vitro* obtained by using human liver microsomes (HLM). Moreover, the luminescent CYP3A4 P450-Glo™ Assay (Promega) was used for testing the effects of the examined compounds on CYP3A4 activity [3]. H_3R ligands were also screened for cytotoxicity against HEK-293 (Human Embryonic Kidney cell line) with use of short term colorimetric EZ4U cell proliferation and cytotoxicity assay protocol. The compounds were examined under various concentrations and compared to the doxorubicin as a standard drug.

Results. The series of metabolites with confirmed by using LC/MS technique molar masses were obtained and compared to the *in silico* results to identify their structural formulas. The antiproliferation effect of H_3R ligands was also characterized and compared to the reference compound doxorubicin.

Conclusion. The most probably metabolic modifications of examined H_3R ligands were defined and included hydroxylation or the degradation of piperidine or azepane moiety followed by oxidation. The compounds were also determinated as a CYP3A4 activators. In comparison to the IC_{50} value of the reference compound doxorubicin, H_3R ligands showed weak antiproliferative effect on HEK-293 cells.

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Acknowledgements
The project was financed from the resources of National Science Centre granted on the basis of decision No DEC-2011/02/A/NR4/00031.

HISTAMINE INTERACTION WITH HISTAMINE H_4 RECEPTOR - A STUDY OF BIOGENIC AMINE MODULATORY EFFECT ON HUMAN EOSINOPHILS FUNCTION

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Background. Histamine is a biogenic signaling amine that contributes to the diverse physiological effects, including immunomodulatory role in inflammation processes. This organic compound acts through four different types of G-protein-coupled proteins, known as histamine receptors. Histamine H_4 receptor is expressed mainly on immune cells, including human eosinophils.
It is believed to play an important role in host defense reaction against external pathogens.

Aim. Regardless of the obtained data the function of H_4 receptor still remains not fully known, although it is postulated that this receptor contributes in regulation of immune cells migration into the site of inflammation. Therefore understanding the physiological role of H_4 receptor might help in search of new drugs that could be used in treatment of chronic inflammatory conditions like asthma or allergy. The aim of the presented study was to estimate the role of histamine and histamine H_4 receptors in the activation process of human eosinophils.

Materials and methods. Human eosinophils were isolated from fresh human blood by Ficoll-Paque density gradient separation, followed by negative immunomagnetic cell sorting, using MACS Miltenyi Biotec system. Viability and functionality tests of the isolated cells were performed based on trypan blue staining and eosinophils peroxydase (EPO) release assay. Histamine effect on eosinophils adhesion to endothelium cells was evaluated during eosinophils co-culture with human HMEC-1 and Ea.hy.926 endothelium cell line, both in static and in flow conditions.

Results. As a result pure eosinophils population was isolated from human blood. The cells were characterized by high viability and functionality, lasting for approximately 3h after isolation. Action of histamine and selective histamine H_4 receptor ligand JNJ7777120 on eosinophils resulted with different eosinophils reactivity and adhesion efficiency to endothelium cells.

Conclusion. In conclusion we hope that the study of histamine interaction with human eosinophils will help to understand the physiological role of human histamine H_4 receptor. This model might also be used in evaluation of newly synthesized histamine H_4 receptor agonists and antagonists.

This project was financed by a grant from the National Science Center based on decision no. DEC/2011/02/A/NZ4/00031.

H_4R ANTAGONIST AND OPIOID RECEPTOR ANTAGONISTS INTERACTIONS IN ANTINOCICEPTION

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Aim. Ligands of histamine H_4 receptor (H_4R) demonstrated analgesic effects in various animal pain models but little is known about the role of this receptor in pain physiology. Aim of this study was to identify mechanisms that may modulate role of H_4R in nociception. Pharmacodynamic interactions between selective H_4R ligand and opioid receptor antagonists with well-described mechanism of action were analyzed.

Materials and methods. Behavioral studies were performed at WAG rats (Cmd/Crl). Male rats weighing 250-300 g were used for experiments. The rats were housed two per cage under natural light-dark cycle with free access to standard food pellets and water. All drugs were applied intraperitoneally. Analyzed agents were: H_4R inverse agonist JNJ7777120 (J77) (20 mg/kg), non-selective opioid receptor antagonist - Naloxone (10 mg/kg), selective opioid receptor antagonists MOR - Naloxonazine (1 mg/kg), DOR - Naltrindol (1 mg/kg), KOR - Bi-Naltrophimine (1 mg/kg).
Pain threshold was assessed at thirty-minute intervals. For a complete evaluation, the measurements of pain threshold three types of nociceptive stimuli were used. Mechanical pain threshold was determined by the method of Randall-Selitto. Sensitivity to acute thermal stimulation was measured in Plantar test method and Tail Flick method.

Results and conclusions. Opioid receptor antagonists, on the one hand, abolished analgesic action of J77 vs. mechanical stimuli on the other hand Naloxone, DOR antagonist and KOR antagonist potentiated antinociceptive action vs. thermal stimuli. Further studies are necessary to analyze this phenomenon. Because various painful stimuli are experienced by different nociceptors, conducted by various fibers, and can be differentially modified by complex regulatory systems. It is probable that H_4R may be differentially expressed on these structures.

Acknowledgments

This project was financed by a grant from the National Science Center based on the decision No DEC-2011/03/N/NZ4/03765. Research subject carried with the use of CePT infrastructure financed by the EU – the European Regional Development Fund within the Operational Program "Innovative economy" for 2007-2013. Mr Rzodkiewicz is a Fellow of Ph.D. Scholarship financed by the EU within Human Capital Operational Programme for 2007-2013.

MAST CELLS - NOT JUST IMPORTANT IN ALLERGY!

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Invited Lecture

This presentation will give a brief history of the mast cell and the well known role of mast cells in allergic diseases. However, mast cells are also key players in innate immunity due to the many receptors on their cell surface, e.g. TLR-2 and -4. Mast cell-derived mediators are important in cell trafficking, antigen presentation, bacterial killing as well as inducing cytokine production and mucus secretion from epithelial cells. The role of mast cells in other diseases will be presented. In cancer mast cells can have both pro- and anti-tumour effects. The realization that mast cells are important in other disease pathways suggests that these cells and their mediators should be the target for novel therapeutic measures.

TLR3- and TLR7-mediated phenotype alterations and interferon type I synthesis as an evidence for mast cell role in viral infections

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Aim. The aim of this study was to examine mast cells (MCs) for constitutive expression of molecules associated with cellular antiviral response as well as to evaluate the effect of TLR3 and TLR7 ligation on MC phenotype and interferon (IFN) type I release.

Materials and methods. All experiments were carried out *in vitro* on freshly isolated fully mature rat peritoneal MCs. Flow cytometry analysis was performed to assess protein expression whilst IFN type I release was evaluated using ELISA method.

Results. We demonstrated that MCs express TLR3 and TLR7 molecules (both were detected intracellularly and on the cell surface) specific for viral dsRNA and ssRNA, respectively, as well as other proteins associated with cellular antiviral response, i.e. IRF3, type I and II IFN receptors and MHC class I. We also found that exposure of MCs to TLR3 ligand poly(I:C) and TLR7 ligand R848 induced transient decrease of TLR3 and TLR7 intracellular expression, respectively, while temporarily upregulated their surface protein level. Also, both ligands transiently upregulated MHC I expression whereas poly(I:C) elevated IFNGR level. Interestingly, observed changes in MC phenotype after 6 h-treatment with TLR3 and TLR7 ligand were followed by strong decline after 12 h, even below control constitutive expression level. Importantly, MCs challenged with poly(I:C) and R848 released IFN-α and IFN-β. We also indicated that poly(I:C)- and R848-induced IFN synthesis was TLR3- and TLR7-dependent, respectively. Moreover, we established that IKK but not NF-κB was engaged in IFN synthesis triggered by TLR3 and TLR7 ligation.

Conclusion. Our findings indicate that MCs can respond to viral dsRNA and ssRNA by altering their phenotype and generating IFN type I, thus be actively engaged in antiviral immune response.

This work was supported by the National Science Centre in Poland (Grant No. 2011/03/N/NZ6/03528) and by the Medical University of Lodz (Grant No. 502-03/6-164-01/502-64-005).

CYSTEINYL LEUKOTRIENE RECEPTOR (CYSTLTR1, CYSLTR2, GPR17) EXPRESSION ON MATURE TISSUE MAST CELLS

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Aim. In this study, rat mature tissue mast cells were assessed for mRNA expression of cysLT receptor type 1 (CYSLTR1), cysLT receptor type 2 (CYSLTR2) and G protein-coupled receptor 17 (GPR17).

Materials and methods. Fully matured tissue rat mast cells were obtained from peritoneal cavities of female albino Wistar rats by peritoneal lavage. We used reverse transcription and polymerase chain reaction (RT-PCR) to study the gene expression of CYSLTR1, CYSLTR2 and GPR17. In addition, the constitutive and induced expression after stimulation with specific ligands, i.e. cysLTs (LTC_4, LTD_4, LTE_4) at final concentrations of 0.1, 1, 10, 100 nM, were analyzed using flow cytometry. Heterodimerization of CYSLTR1, CYSLTR2 and GPR17 receptors was analyzed with co-immunoprecipitation and Western-blot tchnique.

Results. We demonstrated constitutive expression of CYSLTR1, CYSLTR2 and GPR17. CYSLTR2 was predominantly expressed at mRNA level. In contrast, expression of CYSLTR1 and GPR17 was considerably lower. We also indicated that rat mast cells constitutively express CYSLTR1, CYSLTR2 and GPR17. Moreover, we observed that GPR17 receptor is located intracellularly, as well. The comparison of cysLT receptor expressions revealed twofold CYSLTR2 higher expression than CYSLTR1 and GPR17. We also tested whether the cysLTs modulate expression of CYSLTR1, CYSLTR2 and GPR17. We found that incubation of mast cells with LTs resulted in a dose-dependent regulation of all three receptors expression. Stimulation with cysLTs significantly increased expression of CYSLTR1, CYSLTR2 and GPR17. What is more, the higher concentration (100 nM) of LTC_4 and LTE_4 induced almost total loss of the surface CYSLTR2 expression. Surprisingly, cysLTs regulated receptor protein expression, but not mRNA expression. We also assessed formation of leukotriene receptors complexes with anti-CYSLTR1 antibodies and we demonstrated that CYSLTR1 constitutively form heteromeric complexes both with CYSLTR2 and GPR17.

Conclusion. We demonstrated that mature tissue mast cells express the CYSLTR1, CYSLTR2 and GPR17. We also reported that CYSLTR1, CYSLTR2 and GPR17 can form heterodimers. Collectively, our findings suggest a possible mechanism by which cysLTs and heterodimers formation can modulate cysLT receptor expression on mast cells.

PLACENTAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PLACENTA GROWTH FACTOR (PIGF) RECEPTORS IN RELATION TO MATERNAL HEMOGLOBIN CONCENTRATION AND PREGNANCY OUTCOME

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Aim. Hypoxia is a common factor stimulating new blood vessels formation. Enhanced placental vasculogenesis and angiogenesis during pregnancy are therefore observed typically under hypoxic conditions. Insufficient tissue oxygenation may be caused by lowered hemoglobin (Hb) concentration and anaemia might induce vascularity development in the fetoplacental unit. The goal of this study was to determine correlations between placental vascular density (assessed as the mean vascular/extravascular tissular index - V/EVTI), local expressions of VEGF and PIGF receptors (flt-1, flk-1), and the birth weight and maternal Hb concentration.

Materials and methods. The studied material consisted of 43 specimens of term placentas, obtained after normal course pregnancies deliveried at term (37-42 weeks of gestation). For standard morphometric procedures, 3 tissue samples from each placenta (the maternal site) were collected. They were immunostained for analysis of receptors flt-1 and flk-1. V/EVTI was measured, which reflects intensity of vascularization by assessing a total vascular area. Using light microscopy, with computed morphometry for quantitative analysis, the mean expressions of flt-1 and flk-1 were measured in calibrated areas of the placental sections. Nonparametric Mann-Whitney U-test and Spearman's correlation were used to compare the various parameters and their differences between the groups created on the basis of the mean Hb concentration and the birth weight. P value < 0.05 was considered significant.

Results. Among women with lowered Hb concentration, local expression of flt-1 was significantly increased and positively correlated with the average birth weight. The same association was denoted for V/EVTI.

Conclusion. Vasculature adaptive changes in the human placenta in response to a low maternal Hb concentration include increase in the capillary network formation, being positively correlated with the birth weight.

EXPRESSION PROFILE OF KININ-RELATED GENES IN HUMAN RENAL PROXIMAL TUBULE CELLS IN RESPONSE TO AMPHOTERICIN B TREATMENT

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Aim. Kinins are important mediators of inflammation and act at the site of tissue injury. In the periphery, kinins control blood pressure, sodium and water excretion and play an important role in enhanced vascular permeability. Amphotericin B (AmB) is an antifungal agent but the use of AmB is associated with toxic side effects, including nephrotoxicity. The cellular mechanism of AmB action is based on an impairment in the membrane barrier function of cells. Moreover, it can also modulate the immune system. However, the influence of AmB on cells at the molecular level remains still poorly understood. Therefore, the present study aims at the identification of differences in kinin-related gene expression pattern in human renal proximal tubule cells (RPTECs) in response to amphotericin B treatment compared to the control cells.

Materials and methods. Total RNA was extracted from RPTECs using TRIzol reagent (Invitrogen, Carlsbad, CA). The analysis of the expression profile of genes related to the kinin signal transduction pathway was performed using oligonucleotide microarrays of HG-U133A 2.0 (Affymetrix, Santa Clara, CA).

Results. Typing of differentially expressed genes was performed in a panel of 120 transcripts of 64 genes encoding proteins involved in intracellular signaling activated by kinins. The changed expression of 7 transcripts for 5 genes (FOS, JUN, MAP2K1, MAPK3, SHC1) was identified (ANOVA, $p < 0.05$) by a cutoff of at least 1.1-fold change.

Conclusions. The expression changes in kinin-dependent genes may lead to further insights into the molecular mechanism of amphotericin B action. Moreover, these results may help to assess new less toxic AmB forms.

This research was financed by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037 and supported by the grant KNW-2-037/D/4/N.

INDUCTION OF HISTAMINE SYNTHESIS IN Cd11b$^+$ Gr-1$^+$ MYELOCYTES AND ITS IMMUNOLOGICAL ROLES IN A MURINE SYNGENEIC TUMOR MODEL

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Invited Lecture

Accumulating evidence has indicated histamine synthesis in several tumor tissues, although it is controversial how histamine modulates tumor immunity. Several research groups including us demonstrated that histamine is involved in suppression of tumor immunity by acting on the H_2 receptors [1, 2]. On the other hand, Yang et al. recently demonstrated the higher frequency of chemical carcinogenesis in the mutant mice lacking histidine decarboxylase (HDC), indicating the anti-tumor effects of histamine [3]. Our purpose in this study is to identify the source of histamine and to clarify the roles of histamine using a murine syngeneic tumor-bearing model.

We used a syngeneic tumor-bearing model using CT-26 cells and male Balb/c mice (5 weeks of age). Histamine synthesis was induced in the splenocytes derived from the tumor-bearing mice on Day-14 only when they were co-cultured with CT-26 cells. Induction of histamine synthesis was found in the CD4$^+$ or CD8$^+$ T cell-depleted populations and was reproduced when the splenocytes were incubated in the conditioned medium obtained during the co-culture, raising the possibility that some soluble mediators induce histamine synthesis in myelocytes. Flow cytometry analyses indicated that HDC was expressed in CD11b$^+$ Gr-1$^+$ populations of splenocytes, which expanded during the tumor-bearing period.

We found that IFN-γ was also produced in the splenocytes during the co-culture period. A major source of IFN-γ was found to be CD8$^+$ T cells, which express both H_2 and H_4 receptors. IFN-γ production in the co-cultured splenocytes was significantly suppressed by H_1 receptor antagonists, pyrilamine and diphenhydramine, and H_4 receptor antagonists, JNJ7777120 and thioperamide, and was unchanged in the presence of an H_2 receptor antagonist, cimetidine.

These results suggest that a major role of histamine released from CD11b$^+$ Gr-1$^+$ myelocytes is to enhance IFN-γ production in CD8$^+$ T cells by acting on the H_4 receptors.

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RECEPTOR AND NON-RECEPTOR MEDIATED BIOLOGICAL EFFECTS OF L- AND D-LACTATE ON CANCEROUS CELLS IN VITRO.

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The model of the lactate as an active metabolite is a new emerging and attractive concept. The evidence for local signaling function of lactate comprises induction of VEGF and TGFβ, metalloproteinases, enhanced endothelial cell mobility and vascularization, increased collagen synthesis and its post translational modification and deposition, cell proliferation, transcription of genes for proteoglycans, CD44, caveolin-1, Hyal-1 and –2 [1]. Indeed, the present evidence place lactate as a pseudo-hormone coordinating physiological processes on systemic and cellular levels. Recently, L-lactate has been identified as a ligand for hydroxycarboxylic acid receptor 1 (HCA_1; EC_{50} = 4.9 mM) [2, 3] predominantly expressed in a fat tissue and adipocytes where mediates process of lipolysis. HCA_1 presence was also confirmed in kidney, liver, muscles, stomach, brain, heart and intestine. Moreover, lactate was also described as a weak inhibitor of histone deacetylases I/II (HDAC I/II) with IC_{50} = 40 mM and 10 mM for L- and D-lactate, respectively [4]. Presence of an increased extracellular concentration of lactate initiates rapid cellular uptake of lactate facilitated by the proton-coupled monocarboxylate transporters (MCT1-4) and promotion of histones H3 and H4 hyperacetylation, a characteristic feature of transcriptionally active chromatin. Modulation of the activity of mucosal cells of female reproductive tract or gastrointestinal tract by lactate through HCA_1 stimulation or by inhibition of histone deacetylases represents appealing idea to be investigated since these cells are constantly exposed to L/D-lactate of bacterial origin. Metabolic activity of local microbiota accounts for up to 30 mM and 95 mM of L- and D-lactate racemate in vaginal secretions [5] and feces [6], respectively. The objective of the study was to evaluate biological effects of L- and D-lactate, within physiologically relevant lactate concentration present in the cervix mucosa. We used human cervical carcinoma cell lines (HeLa, C33A, CaSki) as the model of epithelial cells lining cervico-vaginal canal and HuT-78 and Jurkat T cell lymphoma cell lines as human immune cells model. Experiments showed that cervical carcinoma cell lines bear HCA_1 and that lactate stimulates MAPK signaling pathway in a receptor dependent manner. Stimulated with lactate, epithelial cells exhibited changes in dynamics of DNA repair and resistance to chemotherapeutic drugs. Immune cells were also found responsive toward lactate. We observed that anti-CD3/CD28 or PMA/Ionomycin activated HuT-78 T lymphocyte cells exposed to 10 - 20 mM D-lactate produced significantly more IL-4 and IL-13. Interestingly, isomer D of lactate, exclusively produced locally by gut or cervo-vaginal microbiota was found to be more potent than L isomer. We also documented observation that expression of IL-4 and IL-13 induced by lactate is associated with process that involves lactate uptake by α-CHCA sensitive monocarboxylate transporters and inhibition of histone deacetylases as visualized by increased acetylation of cellular proteins, particularly histones H3 and H4.

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This work was supported by The National Science Centre of Poland grant 2011/03/B/NZ4/00046 and POIG.01.01.02-10-107/09 "Studies of the molecular mechanisms at the interface the human organism - the pathogen – environmental factors (InterMolMed)".

ANGIOGENIC ACTIVITY OF ASCITIC COMPOUNDS IN THE COURSE OF OVARIAN CANCER

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Aim. The basic spread pattern of ovarian cancer is based on peritoneal invasive implants, which growth is directly associated with the angiogenetic effect. These effect is mainly associated with the presence of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) in ascites. The aim of these study was to assess the impact of ascites produced in the course of ovarian cancer on the angiogenesis.

Material and methods. Peritoneal fluid was collected from patients with advanced ovarian cancer, cancer cells were separated from CD45+ leukocytes. Next, mice were injected intradermally with full cellular suspension together with (a) supernatant or (b) phosphate buffered saline, (c) purified cancer cells suspension or (d) CD45+ leukocytes suspension. The angiogenesis index (AI) was measured after 72 hours. In the supernatant and cellular suspension VEGF and Il-8 were measured.

Results. The highest AI was found in the group of isolated cancer cells suspensions as well in the group stimulated with supernatant. Both VEGF and IL-8 were high in supernatants from ascites rich in cancer cells (>45%). A significant correlation was revealed between IL-8 concentration and AI.

Conclusion. We conclude that ascitic fluid produced in the course of advanced ovarian cancer possesses the angiogenetic properties, which are mostly dependent on activity of cancer cells and enhanced by cooperation with infiltrating leukocytes.

TRANSCRIPTION ACTIVITY OF MELATONIN-RELATED GENES IN ENDOMETRIAL CANCER

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Introduction. The experimental studies suggest that melatonin inhibits initiation and growth of hormone-dependent tumors by interacts with membrane and nuclear receptors. Although most of the studies confirm a hypothesis of an anticancer effect of melatonin, but it is not clear whether these effect occur in endometrial cancer. Estrogens play an essential role in the etiology of endometrial cancer, which is the second of common malignancy of female genital tract in Poland.

Aim. The aim of our study was to evaluate transcription activity of genes encoded melatonin receptors and melatonin-related genes associated with regulation of their activity in endometrial adenocarcinoma.

Materials and methods. Normal human endometria and endometrial cancer were obtained at surgery in the Department of Gynecology, Silesian University Hospital in Katowice. All patients underwent hysterectomy for gynecological disorders, including adenomyosis, uterine leiomyoma, uterine prolapsed and endometrial cancer. The tissues were removed from the resected uterus immediately after surgery, snap frozen and stored until study. The molecular analysis was performed 23 endometrial cancer samples in histopathological grades G1, G2, G3 and 14 normal endometria samples in the control group. The gene expression profile was assigned using oligonucleotide microarrays of HG-U133A (Affymetrix, Santa Clara, CA). For validation of the microarray experiment was evaluated using real time QRT-PCR technique.

Results. From 66 transcripts taking part in the regulation of melatonin receptor activity only 18 ID mRNAs were differential in endometrial cancer samples in comparison to the control at p-value <0,05. These genes were down regulated in cancer tissue samples. The mRNA level of gene RGS4 (*Regulator of G-Protein Signalling 4*) showed statistically significant differences between samples of endometrial cancer in three grades and control group. The QRT-PCR results were analyzed in REST 2009 (Qiagen) and had no statistical significance for the transcription activity of melatonin receptors according to the endogenous β-actin control in endometrial cancer samples.

Conclusions. In this study there was presented a significant down-regulation of melatonin biosynthesis genes and G-coupled coding genes, which expression was directly proportional to the cancer grade advance. The results of this study can be a promising way for to establish new therapeutic approaches in endometrial adenocarcinoma treatment.

This study was supported by grant No. KNW-1-141/P/2/0 and KNW-1-158/K/3/0 from Medical University of Silesia, Katowice, Poland.

SUPPRESSOR GENE EXPRESSION PROFILE IN RPTEC CELLS TREATED WITH AMPHOTERICIN B

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Background. Amfotericin B is a polyene antifungal drug which exhibits strong toxicity, including DNA damage. Suppressor genes participate in regulation of cell damage and apoptosis. Increase in their expression results in growth inhibition and cell division arrest. Amphotericin B exert strong nephrotoxicity which could be caused by DNA damage in renal tubule cells.

Aim. The aim of the study was to determine whether in RPTEC treated by amphotericin B expression profile of suppressor genes has changed.

Methods. RPTEC cells were treated with 0.5 µg amphotericin B per ml of medium. Total RNA was extracted with the use of TRIZOL, according to manufacturer protocol. Gene expression profile was evaluated by oligonucleotide microarray HG-U133A 2 (Affymetrix). Comparative analysis included 2744 ID of suppressor genes mRNA.

Results. Analysis showed 56 differentially expressed genes. 23 genes were up-regulated and 33 were down-regulated in amphotericin B treated cells. Among up-regulated genes were, inter alia, JUN, DAB2 and CDKN2A-cyclin dependent inhibitor 2A. Additionally, up-regulated were genes coding β-actin, tubulin proteins and also gene coding proapoptotic protein (PMAIP1). HSPA1A gene, coding heat shock protein was down-regulated. Decrease of expression was also observed in RND3 gene (coding GTP-ase) and ID4 gene (transcriptional factor which regulates apoptosis).

Conclusion. Amphotericin B changes expression profile of suppressor genes. These results suggest participation of suppressor genes in amphotericin-induced nephrotoxicity.

This research was supported by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037.

INFLUENCE OF AMPHOTERICIN B ON HISTAMINE RELATED GENES IN RPTEC CELLS

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Background. Amphotericin B is an antifungal agent for treating invasive fungal diseases. However therapy with amphotericin B is associated with infusion-related reactions (IRRs) and side effects of nephrotoxicity. Little is known about molecular mechanism underlying observed renal tubular toxicity. One of the suggested mechanisms of amphotericin B-induced nephrotoxicity is persistence of an elevated pro-inflammatory cytokine levels caused by stimulation of pro-inflammatory signalling pathways through Toll-like receptor (TLR) 2.
Biogenic amine - histamine - exerts many biological effects. It acts through four subtypes of G-protein-coupled receptors (H_1 – H_4), expressed in various cell types and tissues. Histamine is synthesised by mast cells and other cell types, including kidney (proximal tubule).

Aim. The aim of this study was to assess influence of amphotericin B on histamine related genes expression in human RPTEC (Human Renal Proximal Tubule Cells) cells.

Methods. RPTEC cells were treated with amphotericin B (0.5 µg AmB/ml of medium). The concentration of drug was selected based on cytotoxicity test. Total RNA was extracted with the use of phenol-chlorophorm method. The expression profile of histamine related genes was appointed using oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Comparative analysis of transcriptomes was performed with the use of GeneSpring 12.0 and PL-Grid platforms. Differentiating genes were considered when $p<0.05$ and $FC>1$.

Results. Comparing to control only one gene was differentiating. In human renal proximal tubule cells amphotericin B caused decrease in histamine receptor H_1 gene (HRH1) expression.

Conclusion. Amphotericin B has weak influence on histamine related genes in RPTEC cells, molecular mechanism of renal tubular toxicity caused by amphotericin B needs further studies.

This research was supported by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037.

PROFILE OF MELATONIN RELATED GENES EXPRESSION IN RPTEC CELLS TREATED WITH AMPHOTERICIN B

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Background. Amfotericin B is a polyene antifungal drug. However, it also exhibits strong toxicity, including DNA damage. The presence of membrane melatonin receptors in kidney cells may indicate repair mechanisms induced by the nephrotoxic effect of amphotericin. Melatonin is a hormone involved in many physiological and pathological process. It acts as a transmitter in metabolic mechanisms, it regulates immune response and influences apoptosis process. Melatonin acts through receptor dependent and independent mechanism. Receptors belong to the G protein-coupled receptor superfamily and are divided into two groups: MT1 and MT2.

Aim. The aim of this study was to evaluate influence of amphotericin B on melatonin related genes expression in human RPTEC (Human Renal Proximal Tubule Epithelial Cells) cells.

Methods. Based on cytotoxicity test results RPTEC cells were treated with 0.5 µg amphotericin B per ml of medium. The extraction of total RNA was performed with the use of phenol-chlorophorm method. The expression profile of 66 genes related to activity of melatonin receptors was appointed using oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Differentiating genes were selected with the use of GeneSpring 12.0 and PL-Grid platforms ($p<0.05$ and $FC>1$).

Results. Analysis showed one differentially expressed gene, comparing to control. RGS4 gene, which expression was decreased, is responsible for regulation of GTP-ase activity of G protein alpha subunit. It was proven that protein product of gene RGS4 can modulate melatonin receptors (MT2) activity.

Conclusion. Effect of amphotericin B on kidney cells could be related to regulatory pathway induced by melatonin.

This research was supported by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037.

INDUCTION OF TNF AND SEROTONIN PATHWAYS IN RPTEC CELLS TREATED WITH AMPHOTERICIN B

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Background. The proinflammatory cytokine - Tumor Necrosis Factor (TNF) acts through two receptors: TNFR1 and TNFR2 present on many cell types, determining pleiotropic character of TNF activity. The neurotransmitter - serotonin (5-hydroxytryptamine, 5-HT) modulates many physiological functions and it acts through seven subtypes of 5-HT receptors. 5-HT is an immunomodulator responsible for, inter alia, blockade of TNF induced signalization. This can be mediated through particular subtypes of 5-HT receptors, in example, 5-HT_{2A} can block TNFR1-induced NFκB activation through activation of protein kinase C (PKC) – dependent signalization. Amphotericin B is still one of the basic antifungal agents used in invasive fungal diseases treatment. Cytokines are responsible for infusion-related reactions (IRRs) and side effects of nephrotoxicity observed in patients treated with amphotericin B. However molecular mechanism of renal tubular toxicity remains unclear.

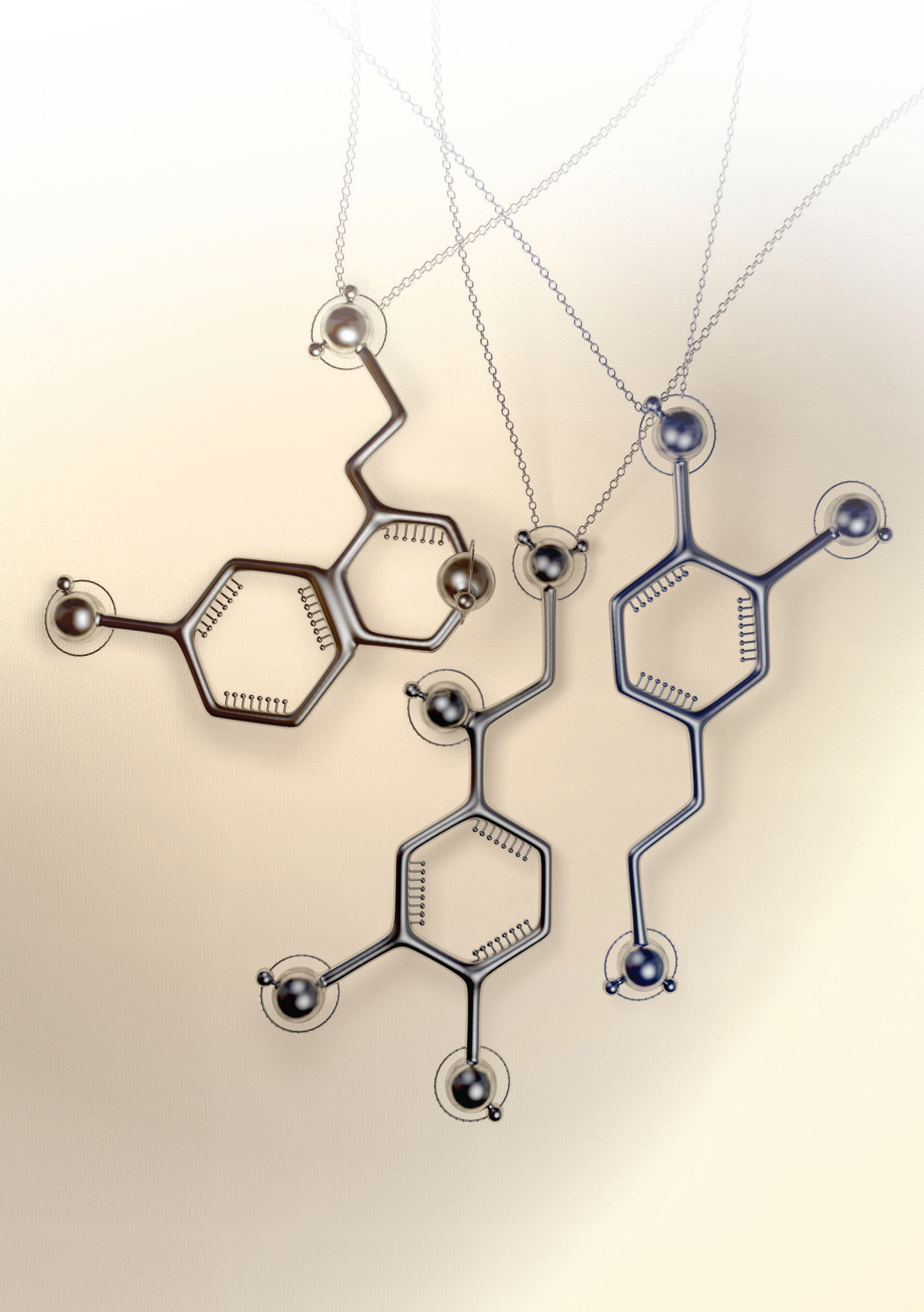
Aim. The aim of this work was to assess the changes in transcriptomes of genes related to TNF and serotonin signal transduction pathways in Human Renal Proximal Tubule Epithelial Cells (RPTEC) after treatment with amphotericin B.

Methods. Based on cytotoxicity test results, RPTEC cells were treated with 0.5 µg amphotericin B per ml of medium. Total RNA was extracted using phenol-chlorophorm method. The expression profiles of genes related to TNF and serotonin signal transduction pathways were determined using oligonucleotide microarrays (HG-U133A 2.0, Affymetrix). Appointment of differentiating genes was performed with the use of GeneSpring 12.0 and PL-Grid platforms, presuming that $p<0.05$ and $FC>1$.

Results. Comparing to control 14 genes were differentiating. Eight of them were upregulated in amphotericin B treated RPTEC cells: JUN, DUSP3, TNFAIP3, DDIT3, CFLAR, MAPK3, PLCB1 and DUSP10. Six genes were downregulated: HSPA1A, HSPA1B, REL, FOS, ZFAND5, PLCB1.

Conclusion. Amphotericin B induces changes in transcriptomes of genes related to TNF and serotonin signal transduction pathways in human renal proximal tubule cells.

This research was supported by the National Science Centre of Poland on the basis of decision no. DEC-2012/05/B/NZ1/00037.



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