XIV Conference
XIV Konferencja

Lodz, Poland
25-27 October 2012

Joint Meeting of the Polish Histamine Research Society & COST ACTION CM 1103
"Structure-based drug design for diagnosis and treatment of neurological diseases: dissecting and modulating complex function in the monoaminergic systems of the brain"

Aminy biogenne
i pokrewne związki
o wysokiej aktywności
biologicznej

# Biogenic Amines and Related Biologically Active Compounds

Dear Colleague,

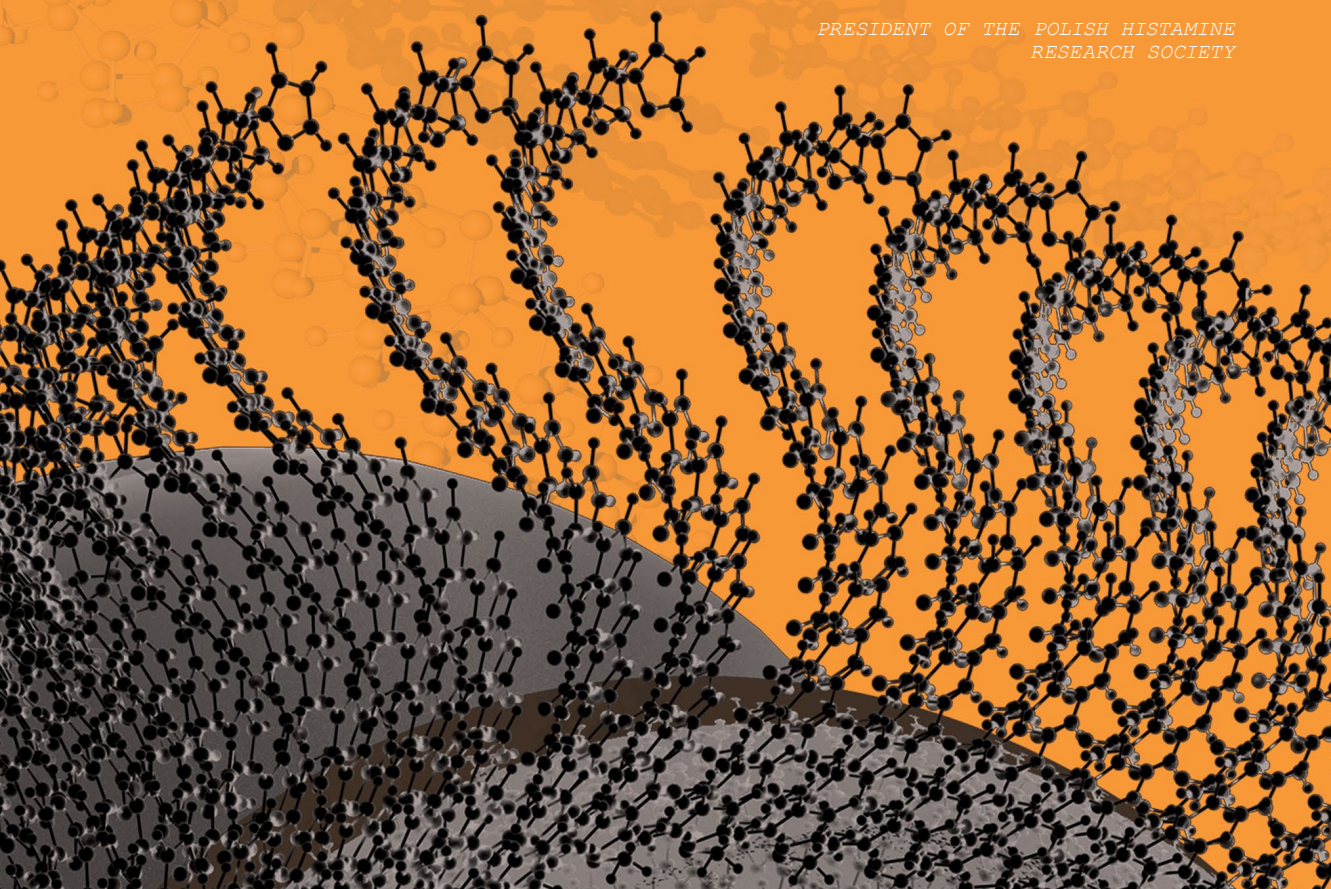
Welcome to the XIVth Conference of the series "Biogenic Amines and Related Biologically Active Compounds". This year again, our meeting is a joint one, bringing together members of the Polish Histamine Research Society and one of the COST Actions, namely CM1103. Due to that we have amongst us several well known European scientists, working in our field of interest.

The content of the presentations covers some hot topics: the most recently Nobel prized G-Protein Coupled Receptors and aging, with neurodegeneration, macular degeneration and some other pathological processes. I am sure that this, together with multidisciplinary approach as participants are basic researchers: biochemists, medicinal chemists, molecular biologists as well as clinicians, will result in a very interesting outcome of the conference.

I wish everybody much satisfaction and joy from presenting or/and discussing their own and other's data and good relaxation while listening to the good music by Arthur Rubinstein Lodz Philharmonic Orchestra on Friday evening.

*W. Agnieszka Fogel*

*PRESIDENT OF THE POLISH HISTAMINE RESEARCH SOCIETY*



# CONFERENCE PROGRAMME

Hotel Ambasador Centrum, Lodz, Poland

## Thursday, 25.10.2012

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

18:00 Opening Ceremony
Prof. W. Agnieszka Fogel,
*President of the Polish Histamine Research Society*

18:15 *Conference Lecture:*
**BIOLOGICAL EVALUATION OF NOVEL MULTIPOTENT MOLECULES (MTDL) DERIVED FROM DONEPEZIL AND PF 9601N DESIGNED FOR THE THERAPEUTIC USE IN ALHZEIMER´S DISEASE**
M. Unzeta, A. Gella, A. Samadi, J. L. Marco-Contelles, I. Bolea,
*Departament de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain; Laboratorio de Radicales Libres y Química Computacional (CSIC), Juan ed al Cierva 3, 28006 Madrid, Spain; Facultat de Ciències de la Salut, Universitat Internacional de Catalunya, Spain*

19:00 Dinner

## Friday, 26.10.2012

9:30-13:00 Session I, chaired by Massimo Valoti and Dariusz Szukiewicz

**INHIBITION OF MAO A BY OLD AND NEW IRREVERSIBLE INHIBITORS,**
J. Allan and R. R. Ramsay,
University of St Andrews, Scotland, UK

**INTERACTIONS OF MAO INHIBITORS WITH CYTOCHROME P450 SYSTEM,**
S. Dragoni, V. Simone, F. Pessina, M. Frosini, M. Unzeta, M. Valoti,
Dipartimento di Neuroscienze, Università di Siena, Italy; Departament de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, Spain

**THE STUDY OF $H_3$ HISTAMINE RECEPTOR LIGANDS DL76 AND DL77 METABOLISM USING IN VITRO MODELS OF BIOTRANSFORMATION,**
G. Latacz, K. Brandowska, K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs, Collegium Medicum Jagiellonian University, Cracow, Poland

Coffee/Tea

**THE $H_4$ RECEPTOR AND INFLAMMATORY CELLS,**
M. Ennis,
Centre for Infection and Immunity, Health Sciences Building, School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, Belfast, UK

**HISTAMINE $H_4$ RECEPTOR ACTIVITY OF STYRYL DERIVATVES OF 1,3,5-TRIAZINE,**
D. Łażewska, J. Ziemba, J. Ner, K. Kamińska, S. Schwed, R. Seifert, H. Stark, K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Institute of Pharmaceutical Chemistry, Biozentrum, Goethe University, Frankfurt, Germany; Department of Pharmacology, Medical School of Hannover, Hannnover, Germany

**PRELIMINARY SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF 1,3,5-TRIAZINE DERIVATIVES,**
K. Kamińska, M. Dutka, M. Zygmunt, S. Mogilski, M. Kubacka, B. Filipek, K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Department of Pharmacodynamics, Jagiellonian University Medical College, Cracow, Poland

**HISTAMINE $H_1$ AND $H_3$ ANTAGONISTS INFLUENCE ACUTE INFLAMMATION IN RATS,**
P. Rzodkiewicz, E. Wojtecka-Łukasik, D. Maślińska, D. Szukiewicz, S. Maśliński,
Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland; Department of General and Experimental Pathology, Medical University of Warsaw, Poland; Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

**NON-IMIDAZOLE HISTAMINE $H_3$-ANTAGONISTS - SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVESTIGATION OF NEW 1-SUBSTITUTED METYL-4-HYDROXYPIPERIDINE DERIVATIVES,**
I. Masłowska-Lipowicz and K. Walczyński,
Department of Synthesis and Technology of Drugs, Medical University of Lodz, Lodz, Poland

**EXPRESSION OF INFLAMMATION-RELATED GENES IN DERMAL FIBROBLASTS AFTER PORCINE ENDOGENOUS RETROVIRUS INFECTION,**
M. Kimsa, B. Strzałka-Mrozik, M. Kimsa, C. Kruszniewska-Rajs, J. Adamska, U. Mazurek,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland

**CROSSTALK BETWEEN TNF AND SEROTONIN INDUCED PATHWAYS IN PORCINE ENDOGENOUS RETROVIRUS (PERV) INFECTION MODEL,**
J. Gola, B. Strzałka-Mrozik, M. Kimsa, M. Kimsa, J. Adamska, C. Kruszniewska-Rajs, U. Mazurek,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland

13:00 Lunch

14:30-17:00 Session II, chaired by Katarzyna Kieć-Kononowicz and Jerzy Jochem

**CHRONIC TREATMENT WITH AMITRIPTYLINE INFLUENCES CARDIOVASCULAR RESPONSIVENESS TO HISTAMINE IN RATS,**
J. Jochem, K. Walas, K. Chojnacka, A. Mitera, A. Krawiec, A. Stasiak, A. Kasperska-Zając, Department of Basic Medical Sciences, Medical University of Silesia, Katowice, Poland; Department of Hormone Biochemistry, Medical University of Lodz, Lodz, Poland; Chair and Clinical Department of Internal Diseases, Allergology and Clinical Immunology, Medical University of Silesia, Katowice, Poland

**MORPHOMETRICAL CHARACTERISTICS OF MICROVASCULATURE AND EXPRESSION OF VEGF AND HISTAMINE RECEPTORS IN PLACENTAE FROM ANEUPLOID PREGNANCIES,**
M. Pyzlak, G. Szewczyk, J. Ostrowska, J. Jędrych, A. Stangret, D. Szukiewicz,
Department of General and Experimental Pathology, Second Medical Faculty, Medical University of Warsaw, Poland; Department of Pathology, Witold Orlowski Clinical Hospital, Center for Medical Postgraduate Education, Warsaw, Poland

**HYPERHISTAMINAEMIA IN THE PLACENTAL COMPARTMENT DURING DIABETES CLASS C MAY BE A RESULT OF BRADYKININ RECEPTOR B1 OVEREXPRESSION,**
D. Szukiewicz, G. Szewczyk, M. Pyzlak, T. K. Mittal, H. Alkhalayla, A. Stangret,
Department of General and Experimental Pathology, Department of Obstetrics and Gynecology, Second Medical Faculty, Medical University of Warsaw, Poland

**UPREGULATED PLACENTAL BETA-DEFENSIN 3 (hBD-3) EXPRESSION IN CHORIOAMNIONITIS (ChA) IS ACCOMPANIED BY INCREASED LOCAL ACTIVITY OF HISTIDINE DECARBOXYLASE (HDC),**
D. Szukiewicz, G. Szewczyk, A. Stangret, H. Alkhalayla, A. Bilska,
Department of General and Experimental Pathology, Department of Obstetrics and Gynecology, Second Medical Faculty, Medical University of Warsaw, Poland

**EXPRESSION PROFILE OF GENES LINKED WITH ADRENERGIC SYSTEM IN ENDOMETRIAL CANCER,**
G. Janikowska, A. Jęda, A. Witek, J. Orchel, S. Dudek, T. Janikowski, U. Mazurek,
Department of Analytical Chemistry, Department and Clinic of Obstetrics and Gynecology, Department of Molecular Biology, Medical University of Silesia, Katowice

**EXPRESSION OF CYTOCHROME P450 GENES ASSOCIATED WITH ADRENERGIC SYSTEM IN ENDOMETRIAL CANCER,**
A. Jęda, G. Janikowska, A. Witek, C. Kruszniewska-Rajs, J. Orchel, T. Janikowski, U. Mazurek,
Department and Clinic of Gynecology and Obstetrics, Department of Analytical Chemistry, Department of Molecular Biology, Medical University of Silesia, Katowice

**HISTAMINE-RELATED GENE EXPRESSION PATTERN IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS,**
B. Strzałka-Mrozik, M. Kimsa, M. Kimsa, K. Michalska-Małecka, C. Kruszniewska-Rajs, A. Kabiesz, W. Romaniuk, U. Mazurek,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; Department of Ophthalmology, Medical University of Silesia, Katowice, Poland

**EXPRESSION PATTERN OF KININ-DEPENDENT GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS,**
K. Michalska-Małecka, B. Strzałka-Mrozik, M. Kimsa, M. Kimsa, C. Kruszniewska-Rajs, A. Kabiesz, W. Romaniuk, U. Mazurek,
Department of Ophthalmology, Medical University of Silesia, Katowice, Poland; Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland

**EXPRESSION PROFILE OF MATRIX METALLOPROTEINASES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS,**
M. Kimsa, B. Strzałka-Mrozik, M. Kimsa, K. Michalska-Małecka, S. Dudek, A. Kabiesz, W. Romaniuk, U. Mazurek,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland; Department of Ophthalmology, Medical University of Silesia, Katowice, Poland

**INFLUENCE OF PACAP38 AND PACAP6-38 ON THE VIABILITY OF Y79 RETINOBLASTOMA CELLS,**
J. Ł. Wojcieszak and J. B. Zawilska,
Department of Pharmacodynamics, Medical University of Lodz, Lodz, Poland

17:00 Sandwich/Coffee/Tea

18:15 Bus transfer to The Arthur Rubinstein Lodz Philharmonic

19:00 Symphony concert: "Notes from the journey", 30 years of artistic work of Krzysztof Kaminski
Krzysztof Kaminski (bassoon) and Arthur Rubinstein Philharmonic Orchestra, conductor: Marcin Wolniewski
*J. N. Hummel - Bassoon Concerto in F major, C. Stamitz - Bassoon Concerto, F. Mendelssohn-Bartholdy - Symphony No. 3 in A minor, Op. 56, "Scottish"*

21:30 Dinner, "U Fabrykanta" Restaurant
Transfer back

## Saturday, 27.10.2012

9:30-12:00 Session III, chaired by W. Agnieszka Fogel and Krzysztof Walczyński

**SEROTONERGIC AND NORADRENERGIC NEURONS SHAPE THE PATTERN OF EXTRACELLULAR DOPAMINE INDUCED BY L-DOPA IN THE PARKINSONIAN BRAIN,**
P. De Deurwaerdère, S. Navailles, L. Milan,
Institut des maladies Neurodégénératives (UMR CNRS 5293), University Bordeaux Segalen, Bordeaux, France

**SEARCHING FOR ANTIPARKINSONIAN DRUGS CONSIDERING DOPAMINE AND ADENOSINE $A_{2A}$ RECEPTOR LIGANDS,**
K. Kieć-Kononowicz, A. Drabczyńska, T. Karcz, C. E. Müller, M. Zygmunt, J. Sapa,
Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; PharmaCenter Bonn, University of Bonn, Germany; Department of Pharmacodynamic, Laboratory of Pharmacological Screening, Jagiellonian University Medical College, Cracow, Poland

**NOVEL AGONISTS FOR THE DOPAMINE $D_3$ RECEPTOR SUBTYPE WITH HIGH IN VIVO ACTIVITY,**
T. Kottke, E. M. Eichelsbacher, N. Bakthiari, J. M. Leppanen, B. C. Sasse, O. Saur, M. P. Hill, A. R. Crossman, E. Bezard, H. Stark,
Institute of Pharmaceutical Chemistry, Goethe University, Biozentrum, Frankfurt am Main, Germany; Department of Pharmaceutical Chemistry, University Kuopio, Kuopio, Finland; Motac Neuroscience Ltd., Manchester, UK; Lab. Neurophysiologie CNRS UMR 5543, Bordeaux, France

**SALSOLINOL AFFECTS GASTROINTESTINAL MAST CELLS VIA LOCAL MECHANISMS,**
M. Kurnik, K. Gil, B. Bujak-Giżycka, J. Madej, A. Bugajski, P. Thor,
Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland; Department of Pharmacology, Jagiellonian University Medical College, Cracow, Poland

**CHANGES IN THE EXPRESSION OF GalR1 AND GalR2 GALANIN RECEPTORS IN THE COLITIS CAUSED BY BRACHYSPIRA HYODYSENTERIAE INFECTION,**
K. Wąsowicz, M. Załęcki, P. Podlasz, M. Chmielewska, K. Łosiewicz, J. Kaleczyc,
Department of Animal Anatomy, University of Warmia and Mazury, Olsztyn, Poland

*Closing Ceremony of the XIV-th Conference of the Polish Histamine Research Society*

*Coffee/Tea*

12:00 Lunch

14:30-17:00 **COST CM1103**

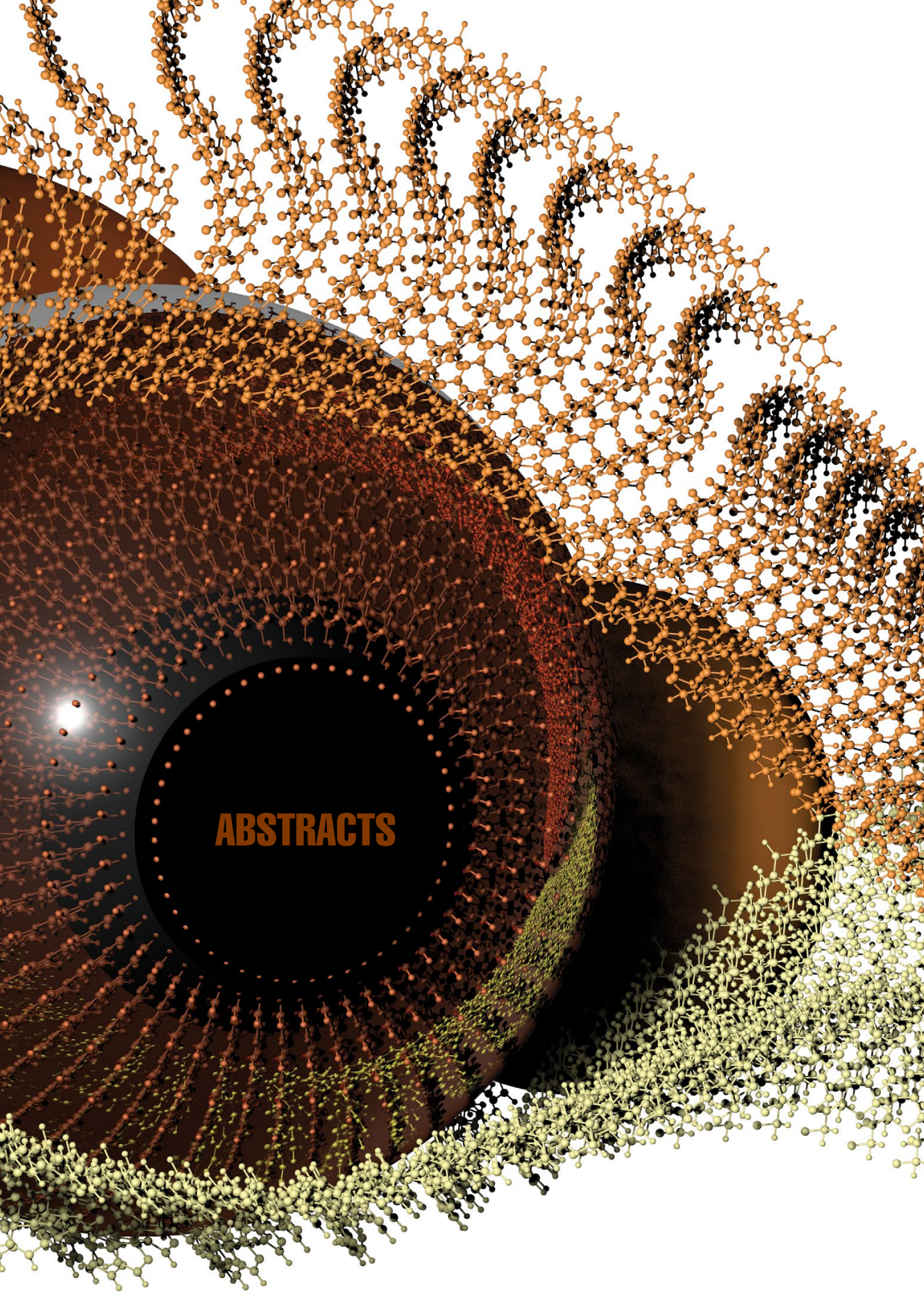
**Holger Stark: Report from WG 1, 2**

Members one-to-one discussion

18:30 **Opera & Dinner**

## Sunday, 28.10.2012

Departure


ABSTRACTS

# BIOLOGICAL EVALUATION OF NOVEL MULTIPOTENT MOLECULES (MTDL) DERIVED FROM DONEPEZIL AND PF 9601N DESIGNED FOR THE THERAPEUTIC USE IN ALHZEIMER´S DISEASE

***Mercedes Unzeta[1], Alejandro Gella[3], Abdelouahid Samadi[2], José L. Marco-Contelles[2], Irene Bolea[1]***

*[1]Departament de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona);*

*[2]Laboratorio de Radicales Libres y Química Computacional (CSIC), Juan ed al Cierva 3, 28006 Madrid Spain;*

*[3]Facultat de Ciències de la Salut, Universitat Internacional de Catalunya, Spain*

The cholinergic hypothesis of Alzheimer's disease (AD) suggests that a selective loss of cholinergic neurons results in a deficit of cholinergic transmission at specific brain regions that mediate learning and memory functions. However, alterations in other neurotransmitter systems, especially serotoninergic and dopaminergic, are also thought to be responsible for the behavioural disturbances observed in AD patients. Moreover, other aspects such as Aβ peptide aggregation and deposition and oxidative stress have also been reported to play a central role in the development of this neurodegenerative disorder. Thus, AD can be defined as a multifactorial disorder that might be more effectively addressed by using drugs able to bind to different type of targets. In this context our aim was to design and synthesize a novel hybrid molecule with a multipotent profile against cholinesterases (ChE) and monoamine oxidases (MAO) and able to inhibit Aβ peptide aggregation.

The newly synthesized molecule, ASS234, showed a potent inhibitory profile able to inhibit both MAO and ChE enzymes (nM range) [1]. ASS234 was also able to prevent the $A\beta_{42}$ self-aggregation (47.8 ± 2.1%) and the AChE-dependent $A\beta_{40}$ aggregation (32.4 ± 7.0%). In addition, ASS234 significantly reduced the Aβ-induced apoptotic death in neuroblastoma cells by preventing caspase-9 and caspase-3 activation as well as blocking the cleavage of PARP. These results indicate that the mitochondrial pathway of apoptosis is involved in the anti-apoptotic action of this compound. Besides the anti-apoptotic properties, ASS234 was also able to restore the Aβ-induced depletion of SOD-1 and Catalase expression and thus showing an anti-oxidant effect. Finally, ASS234 prevented the formation of the toxic oligomeric species of Aβ, suggesting that this may be the mechanism by which it confers neuroprotection in neuroblastoma cells. All together these results demonstrate that ASS234 is a promising multi-target drug candidate with potential to modify the natural course of the disease.

***References***

*1. Bolea I, Juárez-Jiménez J, de Los Ríos C et al. Synthesis, Biological Evaluation, and Molecular Modeling of Donepezil and N-[(5-Benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine Hybrids as New Multipotent Cholinesterase/Monoamine Oxidase Inhibitors for the Treatment of Alzheimer's Disease. J Med Chem 2011; 54: 8251-8270.*

*Jennifer Allan*
*and Rona R. Ramsay*

# INHIBITION OF MAO-A BY OLD AND NEW IRREVERSIBLE INHIBITORS

*University of St Andrews, Scotland, UK*

Clorgyline and deprenyl, potent and effective irreversible inhibitors of monoamine oxidase (MAO) A and B respectively, form a characteristic covalent adduct with the N5 group of the flavin adenine dinucleotide (FAD) co-factor of MAO-A. Two new inhibitors with the same reactive group have been developed as dual inhibitors of MAO and acetyl cholinesterase for use in treating Alzheimer's disease: the N-methylprop-2-yn-1-amine derivative, ASS234 [1] specific for MAO-A and PFN [2] specific for MAO-B were the gift of Prof. J. Marco-Contelles, Madrid, through COST Action CM1103. The binding of these inhibitors to the active site of MAO-A was characterised by steady-state kinetic analysis and visible spectroscopy, and compared with clorgyline and deprenyl.

Without preincubation with MAO, all compounds were competitive inhibitors of amine oxidation, with Ki values of 0.11 μM for ASS234 and 0.78 μM for PFN against purified human MAO-A in agreement with the published selectivity. With 10 min preincubation, ASS234 and clorgyline irreversibly inhibited MAO-A with nanomolar IC50 values, but PFN and deprenyl exhibited over 200-fold lower potency. The rate constants show that ASS234 inactivates the enzyme 4 times faster than clorgyline whereas PFN inactivates MAO-A with the slowest rate. All four inhibitors gave the same spectral change on addition to MAO-A, with a peak at 415 nm consistent with the formation of the N5 adduct. Mass analysis was consistent with this adduct.

**Conclusions.** The new multi-target compounds, are potent irreversible inhibitors of MAO-A (ASS234) and MAO-B (PFN). ASS234 forms an irreversible adduct N5 with MAO-A as efficiently as clorgyline.

### *References*

*1. Bolea I, Juárez-Jiménez J, de Los Ríos C et al. Synthesis, Biological Evaluation, and Molecular Modeling of Donepezil and N-[(5-Benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine Hybrids as New Multipotent Cholinesterase/Monoamine Oxidase Inhibitors for the Treatment of Alzheimer's Disease. J Med Chem 2011; 54: 8251-8270.*

*2. Pérez V, Marco JL, Fernández-Álvarez E, Unzeta M. Relevance of Benzyloxy Group in 2-Indolyl Methylamines in the Selective MAO-B Inhibition. Br J Pharmacol 1999; 127: 869-876.*

# INTERACTIONS OF MAO INHIBITORS WITH CYTOCHROME P450 SYSTEM

*Stefania Dragoni[1], Veronica Simone[1], Federica Pessina[1], Maria Frosini[1], Mercedes Unzeta[2], Massimo Valoti[1]*

*[1]Dipartimento di Neuroscienze, Università di Siena, Italy;*

*[2]Departament de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, Spain*

The determination of metabolic profiles of a new drug gives information that might be used to guide further modifications of a chemical in order to obtain favorable therapeutic properties. In this context the determination of enzyme kinetic for the metabolic reaction can be used for a better estimation of the pharmacokinetic parameters. The goal is not only to identify a single new molecule towards a suitable and selective target but, as soon as possible, the ideal candidates for development must have optimal pharmacokinetic characteristics and toxicological feature. In this context, cytochrome P450 (CYP) plays a crucial role either in metabolism and toxic action of a drug.

Several monoamine oxidase (MAO) inhibitors present a propargylamino moiety. This chemical group confers them properties of irreversible inhibitors towards the MAO and could represent a potential molecular site to the formation of suicide substrates toward CYP. In fact CYP metabolism could give rise to the formation of an active electrophil binding site, resulting in an inhibition of CYPs, which could be responsible of a drug-drug interaction when compounds should be administer in a multiple therapy. For these reasons we have studied the CYP-dependent metabolism and interaction of different propargylamine derivatives with MAO inhibiting properties.

Among the studied compounds L-deprenyl presents an extensive first-pass metabolism which confers it a low oral bioavailability. Moreover the CYP-dependent metabolism is sustained by different isozymes and no irreversible CYPs inhibition was observed. On the contrary the intrinsic clearance of PF9601N, a novel MAO-B inhibitor, was significantly lower than that of L-deprenyl suggesting of an improved bioavailability for the former. Also in this case the CYPs inhibition properties of PF resulted fully competetitive and reversible.

Finally the metabolic features of a series of new PF9601 derivatives, characterized by MAO and acetylcholine esterase (AChE) inhibiting properties were studied. The compounds presented a concentration-dependent inhibition of CYP(s), however this effect resulted fully reversible and a competitive fashion.

In conclusion, all the MAO inhibitors studied showed to be substrates of the CYP isoenzymes with reversible inhibition features. Moreover the propargylamino moiety does not seem to interfere with CYPs at variance with other ethinyl derivatives such as some synthetic estrogens.

*This work was realized in the framework of COST CMST Action CM1103 and working group D34/0003.*

# THE STUDY OF $H_3$ HISTAMINE RECEPTOR LIGANDS DL76 AND DL77 METABOLISM USING IN VITRO MODELS OF BIOTRANSFORMATION

**Gniewomir Latacz, Katarzyna Brandowska, Katarzyna Kieć-Kononowicz**

*Department of Technology and Biotechnology of Drugs, Collegium Medicum Jagiellonian University, 9 Medyczna St., 30-688 Cracow, Poland*

**Aim.** The study of xenobiotic metabolism is a very important part of tests being carried out before distributing the drug to the pharmaceutical market. In this work we used different models of *in vitro* biotransformation to determine the probable direction of phase I metabolism of the antagonists of histamine $H_3$ receptor DL76 (ED50 = 2.8 ± 0.4 mg/kg *7*, hH3R CHO $K_i$ = 22 ± 3 nM) and DL77 (ED50 = 2.1 ± 0.2 mg/kg *per os*, hH3R CHO $K_i$ = 8.4 ± 1.3 nM) [1].

**Materials & Methods.** Biotransformations were carried out with the participation of recombinant and nonrecombinant microbiological models: the strain of fungi *Cunninghamella elegans* DSM 1908, the recombinant yeast cells of *Saccharomyces cerevisiae* W (R) with overexpressing isoform of cytochrome CYP 2B6 and commercial recombinant isoform of cytochrome CYP 3A4.

**Results.** With the application of these models, there were obtained the series of metabolites of DL76 with confirmed by using LC/MS technique two different molar masses and also the series of metabolites of DL77 with confirmed three different molar masses.

**Conclusion.** In conclusion, the LC/MS analysis of the reaction mixtures confirmed significant differences in DL76 and DL77 metabolism between applied recombinant isoforms of cytochrome P450 and fungi *Cunninghamella elegans.* Although that fungi was reported to metabolize a variety of xenobiotics in the ways that are similar to those in mammalian enzyme system, in case of DL76 and DL77 our results showed, that it cannot be considered as an alternative method of biotransformation *in vitro* carried by human isoforms 3A4 and 2B6. The analysis of DL77 biotransformation products obtained by using recombinant isoforms of cytochrome P450 showed the presence of the same metabolite for as well CYP 2B6 as for CYP 3A4. Additionally, the similarity of DL76 metabolism between two applied isoforms of cytochrome P450 was also observed.
A computer simulation of DL76 and DL77 metabolism was also carried out with use of MetabolExpert Pallas 3.1.1.2. program to find a possible structures of obtained metabolites.

### *References*

*1. Łazewska D, Ligneau X, Schwartz JC, Schunack W, Stark H, Kieć-Kononowicz K. Ether derivatives of 3-piperidinopropan-1-ol as non-imidazole histamine H3 receptor antagonists. Bioorg Med Chem 2006; 14: 3522-3529.*

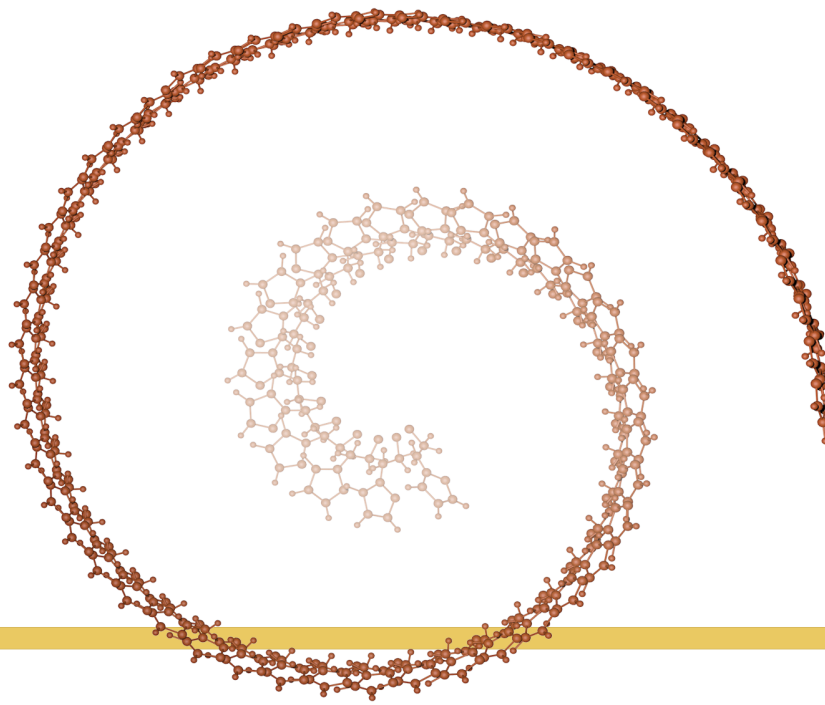
*The work was partly supported by grant: K/ZDS/003325 and COST Action: BM0806.*

# THE $H_4$ RECEPTOR AND INFLAMMATORY CELLS

**Madeleine Ennis**

*Centre for Infection and Immunity,*
*Health Sciences Building,*
*School of Medicine, Dentistry*
*and Biomedical Sciences,*
*Queens University Belfast,*
*97 Lisburn Road,*
*Belfast, UK*

Histamine is an important regulator of biological functions and is best known as an inflammatory mediator in allergic reactions. Histamine mediates its biological effects through four pharmacologically distinct histamine receptors: $H_1R$, $H_2R$, $H_3R$ and $H_4R$. The development of specific agonists and antagonists of the $H_4R$ has allowed the identification of immune responses regulated by the $H_4R$ in eosinophils, mast cells and NKT cells. Although neutrophils are the most abundant leukocytes in blood and play a crucial role in host defence against bacteria and fungi; the role of the $H_4R$ in neutrophil function has been little studied. We have therefore undertaken such a study and this talk will compare our results with neutrophils to the data of others on the eosinophil.



# HISTAMINE $H_4$ RECEPTOR ACTIVITY OF STYRYL DERIVATVES OF 1,3,5-TRIAZINE

***Dorota Łażewska[1], Julia Ziemba[1], Joanna Ner[1], Katarzyna Kamińska[1], Stephan Schwed[2], Roland Seifert[3], Holger Stark[2], Katarzyna Kieć-Kononowicz[1]***

*[1]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, 9 Medyczna St., 30-688 Cracow, Poland;*

*[2]Institute of Pharmaceutical Chemistry, Biozentrum, Goethe University, Max-von-Laue Str.9, 60438 Frankfurt, Germany;*

*[3]Department of Pharmacology, Medical School of Hannover, Carl-Neuberg-Str.1, 30625 Hannnover, Germany*

**Aim**. Histamine $H_4$ receptor ($H_4R$) was discovered in 2000 by several group independently and since that time it has constituted an interesting target for drug development. Results of *iv vitro* and *in vivo* studies suggest that $H_4R$ plays a crucial role in immunological and inflammatory processes [1]. Therefore, the potential utility of histamine $H_4R$ antagonists/inverse agonists could be in the treatment of inflammatory diseases, e.g. allergic rhinitis, asthma, atopic dermatitis, colitis or pruritus. Intensive chemical and pharmacological works have led to potent and selective compounds *e.g.* JNJ7777120, CZC-13788, PF-2988403, INCB38579, UR63325 [2,3]. And the first compound UR 63325 has entered clinical studies for the treatment of allergic respiratory diseases.
Our research group is involved in the search for histamine $H_4R$ ligands and as the continuation the previous work, we prepared the series of styryl derivatives of 1,3,5-triazine and tested them in the binding assay for histamine $H_4R$ [4].

**Materials & Methods.** 1,3,5-Triazine derivatives were synthesized by reaction of 4-methylpiperazine-1-biguanide with the proper methyl cinnamate. Histamine $H_4R$ affinities of compounds were evaluated in the radioligand binding assay, using the model of Sf9 insects cells, transiently expressing recombinant human histamine $H_4R$.

**Results.** Tested compounds showed from weak to good histamine $H_4R$ affinities. The position of the substituent in the styryl part of the molecule influenced the binding to histamine $H_4R$. The highest values were obtained for structures with the substituent in the *meta* position of the phenyl ring. And the most potent compound in this series was 6-(3-chlorostyryl)-4-methylpiperazin-1-yl)-2-amino-1,3,5-triazine with the $K_i$ value of 253 nM.

**Conclusions.** In the considered group of compounds the structural modifications led to obtaining the compound with good histamine $H_4R$ affinity. This compound could be a lead structure for further chemical modifications.

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# PRELIMINARY SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF 1,3,5-TRIAZINE DERIVATIVES

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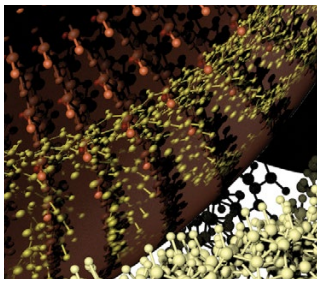
**Aim.** Histamine plays a key role in allergic inflammatory conditions. The most recently identified histamine $H_4$ receptor ($H_4R$) subtype is mainly localized in various cells of the immune system, *e.g.* eosinophils, T-lymphocytes, dendritic cells and basophils [1]. Pharmacological studies suggest that this novel target plays a key role in allergic and immuno-inflammatory disorders [2]. Many different histamine $H_4R$ ligands were described in the literature among them pyrimidine and triazine derivatives [3,4]. The aim of this study was to evaluate anti-inflammtory activity of 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives, selected from the library of compounds synthesized in our department.

**Materials & Methods.** Anti-inflammatory activity of compounds was tested *in vivo* in mice/rats and the ability of these compounds to inhibit the edema induced by carrageenan was measured.

**Results.** The obtained results showed that all tested compounds inhibited edema caused by carrageen in mice. Two compounds TR-7 and TR-18 are going to be also tested in the carrageenan test in rats. The results of these studies will be presented and discussed.

**Conclusions**. The highest anti-inflammatory activity in mice was observed for three compounds KB-20, TR-7 and TR-18.



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*The project was financed from the resources of the National Science Center, granted on the basis of decision No DEC-2011/02/A/NZ4/00031.*

# HISTAMINE $H_1$ AND $H_3$ ANTAGONISTS INFLUENCE ACUTE INFLAMMATION IN RATS

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**Aim.** Histamine receptors are present on cells crucial to inflammatory process such as mast cells, neutrophils, etc. All known antihistaminics may affect several inflammatory events, including chemotaxis and the survival of eosinophils, release of chemokines and cytokines from different sources, thus highlighting the potential for a modulation of inflammation and immune responses. Objective of the project was to evaluate actions of $H_1$-$H_4$ receptor antagonists in acute inflammatory response.

**Materials & Methods.** Inflammation was induced by injection of 12% solution of casein into peritoneal cavity of Wistar rats. Rats were treated intraperitoneally with pyrilamine maleate (histamine $H_1$ receptor antagonist) (10 mg/kg), cimetidine (histamine receptor $H_2$ antagonist) (25 mg/kg), thioperamide maleate (histamine receptor $H_3$/$H_4$ antagonist) (2 mg/kg) and ciproxifan hydrogenmaleate (histamine receptor $H_3$ antagonist) (0.14 mg/kg) twice: 2 hours prior and 4 hours after casein administration. Histamine in whole blood was determined fluorimetrically after chromatography on Dowex 50Wx8 and two stage extraction (n-butanol, n-heptane), using the o-phthaldialdehyde method of Shore. The chemiluminescence assay was performed at 37 $^{o}$C in a final volume of 1 ml PBS supplemented with 0.1% bovine serum albumin and 0.1% glucose containing $10^5$ neutrophils, luminol (150 mM) plus opsonized zymosan (1 mg) and measured as the total light generation after 30 min. The study was approved by the local Ethical Commission.

**Results.** We have confirmed that level of histamine in casein induced inflammation is higher than in control group. Treatment with pyrilamine and ciproxifan additionally increased level of blood histamine during inflammatory response. This data confirm suggestion that stimulation of $H_1$ and $H_3$ receptors by histamine may control the synthesis and releasing of histamine in receptor mediated feedback control system. Some chemokines like substance P which production is mediated through $H_3$ and probably $H_1$ receptors have properties to induce histamine production and release.
Peripheral blood neutrophils from rats with casein-induced inflammation tended to respond less to zymosan stimulation than neutrophils from controls. Selective $H_1$ and $H_3$ antagonists injected to rats with casein induced inflammation significantly increased neutrophils response to zymosan. This observation may be explained by relation between histamine level and reactive oxygen species production. Increased histamine level may influence PMNs activation.

**Conclusion.** It may be concluded that histamine action in the course of experimental casein induced inflammation may be influenced by $H_1$ and $H_3$ receptor antagonists.

# NON-IMIDAZOLE HISTAMINE $H_3$-ANTAGONISTS - SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVESTIGATION OF NEW 1-SUBSTITUTEDMETYL-4-HYDROXYPIPERIDINE DERIVATIVES

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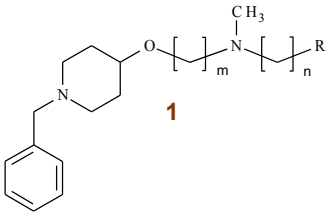
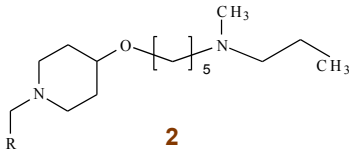



**1**




**2**

**Background.** The cloning of the $H_3$ receptor has provided fresh impetus to the development of drug-like ligands of this receptor. During the years following number of $H_3$ antagonists belonging to different chemical classes, subsequently divided between classical imidazole-based and non-imidazole series have been described; the imidazole derivatives were considered to be less attractive for pharmacokinetic as well as for toxicological reasons. The successful replacement of the imidazole moiety with piperidine and other basic tertiary amines was demonstrated with a variety of analogs. As might have been expected the effect of replacement of the imidazole by basic tertiary amines affected $H_3$ inhibitor potency to different degree, depending on the chemical series. Based on literature data it may be concluded that compounds carrying on piperidine ring are more likely to be successful in ethereal analogs· than in the other series.

**Methods.** In the present work, we report the synthesis and preliminary pharmacological investigation (functionally on *in vitro* test system using guinea pig jejunum preparations) of new series of: 1-benzyl-4-hydroxypiperidines **1** and 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)- pentyloxy]piperidines **2** as $H_3$ histamine receptor antagonists.

**Results & Conclusion.** The presented 1-benzyl-4-hydroxypiperidine **1** and 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)- pentyloxy]piperidine **2** derivatives all possess, moderate to pronounced $H_3$-receptor antagonist activity. All compounds of 1-benzyl-4-hydroxypiperidine series [1], showed weak to moderate $H_3$-receptor antagonist potency. The highest potency for these homologous series is seen in the compound with the *N*-methyl-*N*-propylaminopentyloxy substituent. This derivative was used as a new lead compound for further structural modification of phenyl moiety.

Therefore, a series of 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)pentyloxy]piperidines was synthesized and pharmacological evaluated. The benzo ring was replaced by chromanyl, chromanonyl, benzofuranyl, indenyl and napthanyl moiety. For this series the highest affinity possessed derivatives of 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)pentyloxy]piperidine carrying on benzofuranyl subsituents.

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# EXPRESSION OF INFLAMMATION-RELATED GENES IN DERMAL FIBROBLASTS AFTER PORCINE ENDOGENOUS RETROVIRUS INFECTION

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**Aim.** The therapeutic usage of porcine material in xenotransplantation is not confirmed to be completely safe because of the presence of various pathogens such as porcine endogenous retroviruses (PERVs) and transmission risk to humans. Histamine can play a pathophysiological regulatory role in cellular events and can modulate immune-inflammatory responses. Therefore, the present study focuses on the identification the effect of porcine endogenous retroviruses (PERVs) on human dermal fibroblasts by investigating the changes of the aspect of the expression of many cytokines and histamine receptors.

**Materials & Methods.** The PERV infectivity was analyzed in the co-culture system. Normal human dermal fibroblasts (NHDF cell line) were co-cultured for 5 days with normal porcine kidney epithelial cells (PK15 cell line). Genomic DNA was isolated from harvested cells using the salting out extraction method. Total RNA was extracted from cells using TRIzol reagent. The infectivity of PERVs was determined using real-time Q-PCR and QRT-PCR assay. The analysis of the expression profile of cytokines and histamine receptors was performed using oligonucleotide microarrays HG-U133A 2.0.

**Results.** The copy number of PERV A and PERV B RNA in NHDF cells (637.30 ± 363.0; 77.00 ± 58.0, respectively) were revealed after co-culture. However, only the copy number of PERV A DNA was observed (10.14 ± 7.6). The study indicated that after PERV infection, NHDF cells showed a statistically significant increase of expression of *HRH4* and *IFNG, IL1B, IL6, IL32*, *TNFRSF25*. The down-regulated transcripts were found for 4 genes (*HRH1* and *IL1R1, IL6ST*, *IL33*).

**Conclusion.** PERV infection may alter expression of inflammation-related genes. This can be linked with the integration of PERV retroviral cDNA into the human cell genome. PERVs may be also factor engaged in the generation of inflammatory response.

*This study was supported by the grant No. KNW-1-009/D/2/0 from Medical University of Silesia, Katowice, Poland.*

# CROSSTALK BETWEEN TNF AND SEROTONIN INDUCED PATHWAYS IN PORCINE ENDOGENOUS RETROVIRUS (PERV) INFECTION MODEL

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**Background.** Tumor necrosis factor (TNF) is one of the most important proinflammatory cytokines, responsible for the initiation and maintenance of host defence, during both microbial and viral infections. TNF is secreted mainly by monocytes, macrophages and other cells i.e. fibroblasts. It acts through two receptors: TNFR1 and TNFR2 present on many cell types, determining pleiotropic character of TNF activity. Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter responsible for many physiological functions (*i.e.* sleep, appetite, mood). These responses to serotonin are mediated through seven subtypes of 5-HT receptors. Recently 5-HT has been described as an immunomodulator, responsible for modulating cytokine and chemokine production. One of this immunomodulator effects is blockade of TNF induced response, which can be mediated through different subtypes of 5-HT receptors. For example, $5\text{-HT}_{2A}$–induced activation of protein kinase C (PKC) can block TNFR1-induced NFκB activation. It was also showed that even in the presence of lipopolysaccharide (LPS), serotonin downregulates TNF expression.

**Aim.** The aim of this work was to describe the changes in the transcriptome of the genes related to TNF and serotonin signal transduction pathways stimulated with LPS in PERV-infected human fibroblasts.

**Methods.** Human fibroblasts (NHDF) were co-cultured with normal epithelial porcine kidney cells (PK15 cell line) in the presence of lipopolysaccharide (LPS). Total RNA was extracted with the use of phenol-chlorophorm method. The expression profile of genes related to the TNF and 5-HT signal transduction pathways was appointed with the use of oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Data analysis was performed with the use of GeneSpring 12.0 platform (Agilent Technologies). Genes were considered differentiating when p</=0.05 and FC>/=1 (fold change).

**Results.** Nine genes were differentiating: PPP5C, ATP7A, SLC6A4, GPM6B, MPPE1, SCAMP5, GLUL, SH3TC2 and ANKS1B. Except PPP5C gene, differentiating genes were related to 5-HT. In PERV- infected fibroblasts the most upregulated gene was GPM6B (FC = 2.05) comparing to control.

**Conclusion.** There are small changes in the transcriptome of the genes related to TNF and serotonin signal transduction pathways stimulated with LPS in PERV-infected human fibroblasts. In the future the research concerning changes after stimulation with TNF and 5-HT should be undertaken.

*This study was supported by the grant No. 12 0036 06/2009 (KNW-6-373/09) from Medical University of Silesia, Katowice, Poland and funded by The National Centre for Research and Development (NCBiR).*

# CHRONIC TREATMENT WITH AMITRIPTYLINE INFLUENCES CARDIOVASCULAR RESPONSIVENESS TO HISTAMINE IN RATS

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*Katarzyna Chojnacka[1],*
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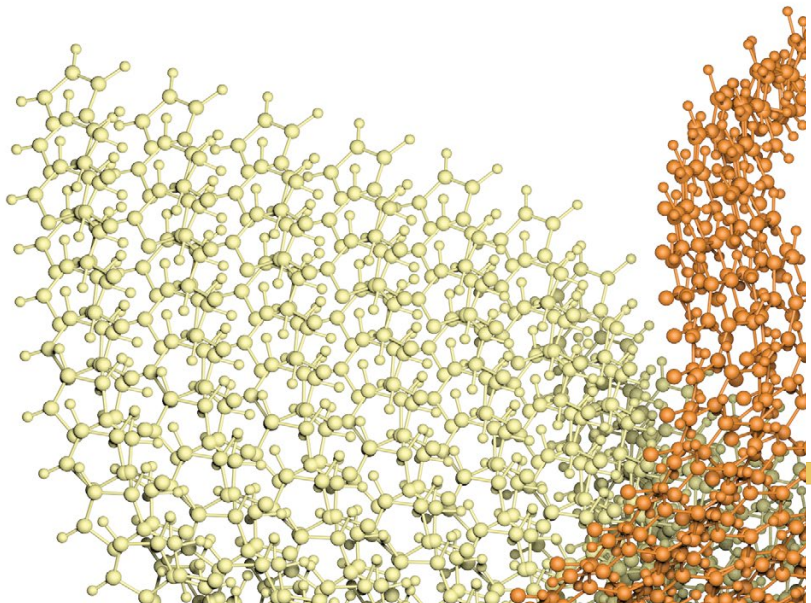
Amitriptyline is a tricyclic antidepressant frequently used in the treatment of many psychiatric, neurological and gastrointestinal diseases. The drug was reported to affect the cardiovascular system functions.
The aim of the study was to examine the influence of chronic treatment with amitriptyline on cardiovascular responsiveness to histamine. Studies were performed in male Wistar rats pre-treated with amitriptyline (2.7 and 5.4 mg/kg, subcutaneously for 7 days). Histamine (5.0 and 10.0 μg/kg) administered intravenously in a bolus injection evoked dose-dependent decreases in systolic and diastolic blood pressure as well as in renal (RBF) and skeletal muscle microcirculatory flow (SMMF), with no significant changes in heart rate (HR) in the control, saline pre-treated group. Amitriptyline inhibited histamine-evoked decreases in blood pressure (2.7 and 5.4 mg/kg), produced increases in RBF and SMMF (5.4 mg/kg), and did not influence HR dynamics.
In conclusion, chronic treatment with amitriptyline affects cardiovascular responsiveness and peripheral perfusion changes after intravenous histamine administration in rats.



# MORPHOMETRICAL CHARACTERISTICS OF MICROVASCULATURE AND EXPRESSION OF VEGF AND HISTAMINE RECEPTORS IN PLACENTAE FROM ANEUPLOID PREGNANCIES

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**Aim.** It is estimated that 50 to 70% of early miscarriages are associated with chromosomal abnormalities, of which over 50 % are constituted by trisomies. Changes in vascular density are indicated as the most important factor in development of fetal trisomy – associated placental pathologies. Thus, factors influencing placental vascular development are of major importance for understanding pathophysiology of placental changes. The aim of the study was the comparison of morphometrical differences in microvasculature and vascular endothelial growth factor receptors type 1 (VEGFR1), type 2 (VEGFR2) and histamine receptor type 1 (H1R) expression in placentae from pregnancies complicated with fetal trisomy 18 and 21 versus pregnancies which showed no fetal chromosomal alterations.

**Materials & Methods.** We analyzed 34 samples of placental villous tissue collected after spontaneous abortions between 19 and 25 weeks of gestation. Placental tissues from women whose pregnancies were complicated by trisomy 21 (n = 14) and trisomy 18 (n = 8) were compared with samples collected from patients whose pregnancies showed no chromosomal abnormalities (n = 12). Immunohistochemistry staining was done with the use of rabbit polyclonal antibody to VEGFR1 (Abcam), rabbit polyclonal antibody to VEGFR2 (Abcam) and rabbit polyclonal antibody to H1R (Affininty Bioreagents). Vessels walls were identified with antibody anti-CD31. Next, morphometrical analysis was performed with the use of Leica imaging workstation and following parameters were calculated: vessels density (number of vessels/ $mm^2$), vessels area/total villous area, VEGFR1 expression, VEGFR2 expression, H1R expression. The mean expression of the following VEGFR1, VEGFR2, H1R was estimated according to the equation: area of colour reaction x intensity of the staining.

**Results:** Tissue samples from pregnancies complicated by trisomies showed significantly lower vascular density (vessels/mm2): 783 +/- 258 for trisomy 21, and 339 +/- 23 for trisomy 18 when compared to 812 +/- 151 (mean +/- SD), p = 0.04 and 0.03 respectively. Vascular density in trisomy 18 was also significantly lower than in trisomy 21 (p = 0.02). Percentage of area taken by blood vessels was significantly lower in aneuploid pregnancies than in euploid ones: 14 +/- 0.5 (p = 0.02) for trisomy 21 and 10 +/- 0,2 (p = 0.026) when compared to 22 +/- 1 for euploid pregnancies (mean percentage +/- SD). Vascular expression of VEGFR-1 was significantly higher in groups of aneuploid pregnancies. while VEGFR-2 expression within endothelial cells was lower in aneuploid pregnancies. Trophoblast expression of H1R were significantly higher in aneuploid pregnancies, and there was no staining for H1R within endothelial cells.

**Conclusion.** Defective placental angiogenesis in trisomy 21 and trisomy 18 is observed together with increased expression of VEGFR1 and H1R.

# HYPERHISTAMINAEMIA IN THE PLACENTAL COMPARTMENT DURING DIABETES CLASS C MAY BE A RESULT OF BRADYKININ RECEPTOR B1 OVEREXPRESSION

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**Aim.** Excess of both histamine and bradykinin have been found in diabetic placental tissue. These well-known mediators of inflammation are supposed to be working together in pathologic environment. Bradykinin may stimulate placental mast cell degranulation, increasing the local histamine levels. Bradykinin receptor type 1 (B1) shows involvement in the inflammatory response, whereas the type 2 receptor (B2) mediates most of the effects induced by kinins. Here we examined comparatively (diabetes class C after White vs normal pregnancy) correlations between placental histidine decarboxylase (HDC) activity, local histamine concentration, and placental bradykinin receptors B1 and B2 expression within the corresponding tissue.

**Materials & Methods.** Placentae have been collected after diabetic pregnancies (N = 14; Group I) and after normal gestations (controls; N = 14; Group II). The measurement of HDC activity in placental samples obtained in a standardized manner was performed using a modified method of Endo. Histamine concentration in placental excisions was estimated fluorimetrically, and the mean expressions of B1 and B2 were examined after immunostaining of the paraffin sections, using quantitative morphometry in the visual fields matched in mean vascular density.

**Results.** Mean HDC activity in diabetic placenta was significantly ($p < 0.05$) increased compared to controls ($3.85 \pm 0.27$ *vs* $2.25 \pm 0.14$ nmol/h/g ± SEM). Histamine concentration was also significantly increased in diabetes ($379 \pm 24.8$ *vs* $236 \pm 14.7$ ng/g of wet weight ± SEM; Group I and Group II, respectively). Mean expression of the B1 was strenghtened in diabetes and reached 292.3% of the value observed for Group II ($p < 0.05$). Mean expression of B2 receptors was similar in both studied groups.

**Conclusion.** Augmented production of histamine in result of HDC hyperactivity may be responsible for some of the pathophysiologic events reported in diabetic materno-placento-fetal compartment. HDC-dependent histamine elevation may modify in situ vascular properties by significant modulation of bradykinin receptors expression. Proinflammatory changes mediated via B1 should be expected rather, than modified vasomotor reactivity related to B2. The role of histamine and kinins in the local angiogenesis should also be considered.

# UPREGULATED PLACENTAL BETA-DEFENSIN 3 (HBD-3) EXPRESSION IN CHORIOAMNIONITIS (CHA) IS ACCOMPANIED BY INCREASED LOCAL ACTIVITY OF HISTIDINE DECARBOXYLASE (HDC)

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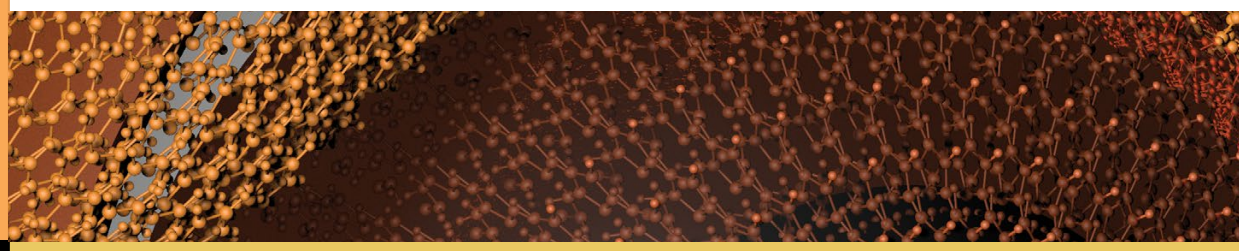
**Aim.** Human β-defensin 3 (hBD-3) is a peptide widely expressed in placenta and the membranes (amnion, chorion) with potent antimicrobal activity. It was suggested recently, that the hBD-3 may stimulate histamine production and release from placental mast cells, acting as pro-inflammatory agent. Intrauterine inflammatory condition called chorioamnionitis (ChA) increases local concentration of hBD-3. In this study we determined relationship between hBD-3 expression and mean activity of histidine decarboxylase (HDC) in normal placenta vs. placenta obtained after ChA-complicated pregnancy.

**Materials & Methods.** Nine placentas obtained after term pregnancies complicated by histologically confirmed ChA were compared with nine placentas obtained from gestationally matched normal-course controls (Groups I and II, respectively). Tissular activity of HDC was assayed in placental cuts collected in standardized manner using modified Endo's method. The same sample collection protocol was used for the study of hBD-3 expression. After immunostaining, hBD-3 expression was examined in 5 μm paraffin sections by computed morphometry for quantitative analysis.

**Results.** The hBD-3 expression was significantly augmented ($p<0.05$) in Group I and amounted to 185.74% ± 16.11 (mean ± SEM) of the value calculated in Group II (controls) taken as 100%. Mean HDC activity within placental tissue was positively correlated ($p<0.05$) with hBD-3 expression (nmol/h/g ± SEM: 4.48 ± 0.31 *versus* 2.13 ± 0.12; Groups I and II, respectively).

**Conclusion.** Augmented hBD-3 expression, accompanied by increased local activity of HDC (thus, hyperhistaminaemia) in ChA-affected placenta, produce both potentially hazardous and beneficial conditions by upregulation of the inflammatory response.



# EXPRESSION PROFILE OF GENES LINKED WITH ADRENERGIC SYSTEM IN ENDOMETRIAL CANCER

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*Joanna Orchel[3],*
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Pathomechanism of endometrial cancer is not fully understood. This estrogen-dependent tumor originates from a single layer of epithelial cells and in the most cases is adenocarcinoma. Its differentiation and aggressiveness depend on the presence and profile changes in concentration of the estrogen receptor isoforms as well as estrogens. Effect of catecholamines on the development of this cancer is connected through hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes. Therefore, assessment of expression profile of genes linked with adrenergic system may be useful to explain the pathogenesis of endometrial cancer.

The intraoperatively taken endometrium was homogenized and total RNA was isolated by the modified method of *Chomczynski et al.* The extract was evaluated spectrophotometrically. After histopathological analysis the seven control and thirteen endometrial adenocarcinoma samples were selected to HG-U133A microarrays procedure which were performed according to the Affymetrix protocol.

From 22283 Affymetrix ID of transcripts were separated of 234 linked with adrenergic system. Profile of expression genes linked with adrenergic system in endometrial adenocarcinoma compared to controls showed a different expression depending on the cancer severity. Among the genes which are changing expression in dependence on grading of differentiation the endometrial cancer are present adrenergic receptors, as well as ADRA2A and ADRA2C. These receptors play a important role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system. The subtype of ADRA2A inhibited transmitter release and ADRA2C modulated neurotransmission.

Reduced levels of these receptors in investigated endometrial cancer compared to normal endometrium may indicate engagement of the adrenergic system in uterine carcinogenesis.

*This study was supported by the grant No. KNW-1-009/P/2/0 from Medical University of Silesia, Katowice, Poland.*

# EXPRESSION OF CYTOCHROME P450 GENES ASSOCIATED WITH ADRENERGIC SYSTEM IN ENDOMETRIAL CANCER

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The cytochrome P450s (CYPs) are a large family of enzymes involved in the biotransformation of substances stimulating or inhibiting the adrenergic system. Members of CYP family are also involved in estrogen metabolism. In both systems, the activity of these substances is regulated by genes such as CYP1A1, 1A2, 1B1 and 3A5. The endometrial cancer mainly concerns women after the menopause, when the hypothalamic-pituitary- gonadal axis is disrupted. The aim of our study was to evaluate expression of cytochrome P450 genes associated with the adrenergic system in different pathomorfological stages of endometrial cancer.

Twenty four human endometrial specimens obtained from patients treated in the Department and Clinic of Gynecology and Obstetric, the Medical University of Silesia, Katowice, were studied. Total RNA was extracted from endometrial specimens using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Total RNA concentration was determined by spectrophotometric measurement using the Gene Quant II RNA/DNA Calculator. Gene expression of cytochrome P450s gene were evaluated using real-time qRT-PCR. Statistical analyses were performed using Statistica 10.0 software, and the level of significance was set at $P<0.05$.

The mRNA level of CYP1B1 showed statistically significant differences between normal and adenocarcinoma in endometrium. The gene expression of CY1B1 decreased with the advancement stage of the cancer in every analyzed case of endometrial cancer.

The received results suggest that the CYP1B1 gene can play the main role in endometrial carcinogenesis. The evaluation of cytochrome P450 gene expression in normal and cancer tissue could become a potential therapeutic target.

*This study was supported by the grant No. KNW-1-016/D/2/0 from Medical University of Silesia, Katowice, Poland.*

# HISTAMINE-RELATED GENE EXPRESSION PATTERN IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS

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**Aim.** Age-related macular degeneration (AMD) is a disease affecting the central regions of the retina and choroid that can lead to irreversible central vision loss. It is caused by multiple factors, such as ageing, genetic predisposition, environmental elements, oxidative stress, and inflammatory effects. Histamine modulates a variety of immune responses, including allergic reactions, as well as the production of antibodies, complements and inflammatory cytokines. Histamine might also have the ability to induce angiogenesis. However, the mechanism by which histamine promotes these responses has not been clarified in detail. The purpose of this study was to assess whether expression of histamine-related genes is altered in peripheral blood mononuclear cells (PBMCs) of patients with wet age-related macular degeneration.

**Materials & Methods.** Total RNA was extracted from PBMCs using the TRIzol reagent (Invitrogen, Carlsbad, CA). The use of oligonucleotide microarray technique HG–U133A (Affymetrix, Santa Clara, CA) enabled an expression level comparison of genes associated with histamine in AMD patients in relation to control subjects.

**Results.** Fluorescence signals analysis of 239 probes, which represented the expression of 121 genes selected from the NetAffx Analysis Center database, demonstrated the differential expression of 72 genes (t test, $p < 0.05$) by an arbitrary cutoff of at least 2-fold change. The increased expression of 32 histamine signaling pathway genes, including *HRH1, HRH3* and *HRH4*, and inhibition of 40 histamine-dependent genes in case of AMD patients compared to the control group were observed.

**Conclusions.** Histamine-related gene determination may help in the identification of various physiologic and pathologic conditions, including ocular diseases. These findings contribute to the clarification of molecular mechanisms involved in the interactions between histamine and AMD disease. Histamine receptor antagonists may also have important therapeutic values.

*This study was supported by the grant No. KNW-1-009/P/2/0 from Medical University of Silesia, Katowice, Poland.*

# EXPRESSION PATTERN OF KININ-DEPENDENT GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS

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**Aim.** Kinins are important mediators of inflammation. Moreover, they play an important role in enhanced vascular permeability and stimulate the process of angiogenesis by activating a vascular endothelial growth factor (VEGF). In turn, inflammation and angiogenesis have been implicated in the pathogenesis of age-related macular degeneration (AMD). Our understanding of the molecular mediators of this disease may influence the treatment of AMD patients. The present study aims at the identification of differences in kinin-dependent gene expression patterns in neovascular AMD patients compared to the control subjects.

**Materials & Methods.** Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) 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 (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 37 genes was identified (t test, $p < 0.05$) by an arbitrary cutoff of at least 2-fold change. The overexpression of 12 kinin-related genes and inhibition of 25 genes were demonstrated in AMD patients.

**Conclusions.** The expression changes in kinin-dependent genes may lead to further insights into their role in the pathogenesis of age-related macular degeneration. The signaling pathways of kinin receptors seem to be promising therapeutic aims.

*This study was supported by the grant No. KNW-1-009/P/2/0 from Medical University of Silesia, Katowice, Poland.*

# EXPRESSION PROFILE OF MATRIX METALLOPROTEINASES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF AGE-RELATED MACULAR DEGENERATION PATIENTS

*Małgorzata Kimsa[1], Barbara Strzałka-Mrozik[1], Magdalena Kimsa[1], Katarzyna Michalska-Małecka[2], Slawomir Dudek[1], Adam Kabiesz[2], Wanda Romaniuk[2], Urszula Mazurek[1]*

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**Aim.** Metalloproteinases (MMPs) with their proteolytic activities participate in cellular homeostasis, adaptation, migration, adhesion, tissue remodeling and angiogenesis. Some key inflammatory agents, including histamine, have been reported to stimulate increase of interleukin and matrix metalloproteinase levels. Both components may be implicated in the pathogenesis of age-related macular degeneration (AMD). Therefore, this study focused on the expression profile of matrix metalloproteinases in peripheral blood mononuclear cells (PBMCs) of AMD patients compared to the control subjects.

**Materials & Methods.** Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen, Carlsbad, CA). The analysis of expression profile of MMP genes was performed using commercially available oligonucleotide microarrays of HG-U133A (Affymetrix, Santa Clara, CA). Typing of differentiating genes was performed in a panel of 43 transcripts of 26 genes encoding MMPs and tissue inhibitor of metalloproteinases (TIMPs).

**Results.** Among all studied genes, 8 expressed more than 2-fold change in the AMD patients (t test, $p<0.05$). 7 genes (MMP12, MMP14, MMP16, MMP19, MMP26, MMP27 and TIMP3) were up-regulated, whereas only one gene (TIMP1) were down-regulated in AMD patients in relation to controls.

**Conclusions.** The molecular details of MMP expression changes may lead to further insights into the role of these proteins in the pathogenesis of AMD and may provide guidelines for the development of novel treatment strategies of ocular disease.

*This study was supported by the grant No. KNW-1-009/P/2/0 from Medical University of Silesia, Katowice, Poland.*

# INFLUENCE OF PACAP38 AND PACAP6-38 ON THE VIABILITY OF Y79 RETINOBLASTOMA CELLS

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**Aim.** In this study we examined effects of VIP and three forms of PACAP (PACAP38, PACAP27, PACAP6-38), alone or in combination, on the viability of Y79 retinoblastoma cell line, the most prominent continuous cell line established from retinoblastoma.

**Materials & Methods.** Cell viability and mitochondrial function were measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction to MTT formazan by cellular mitochondrial dehydrogenases following 24, 48 and 72 h incubation with the peptides.

**Results.** Incubation of Y-79 cells with VIP (0.001 - 1 μM) did not alter their survival. PACAP38 and PACAP6-38 (0.1 – 5 μM) decreased viability of Y-79 cells in a concentration-dependent manner. The cytotoxic effect of both peptides was additive. Exposure of Y-79 cells to PACAP27 also reduced their survival, but the observed effect was markedly weaker than those evoked by PACAP38 and PACAP6-38.

**Conclusions.** It is suggested that cytotoxic effects of PACAP38 and PACAP6-38 on Y79 cells result from their interactions with splice variants of $PAC_1$ receptor in Y79 cells or with another, non $PAC_1$, receptor expressed by these cells.

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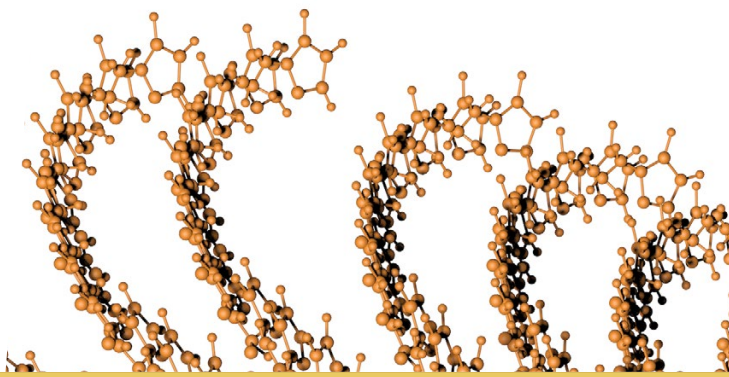

# SEROTONERGIC AND NORADRENERGIC NEURONS SHAPE THE PATTERN OF EXTRACELLULAR DOPAMINE INDUCED BY L-DOPA IN THE PARKINSONIAN BRAIN

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**Aim.** The therapeutic benefit of L-DOPA is commonly attributed to restoration of dopamine (DA) extracellular levels in the striatum of Parkinsonian patients. The DA effects of L-DOPA overwhelm the striatum favouring the idea that the other monoaminergic neurons, serotonin (5-hydroxytryptamine, 5-HT) and noradrenalin (NA), that have a wider distribution in the brain, participate to the release of DA induced by exogenous L-DOPA. We sought to determine in vivo the involvement of NA and 5-HT fibres in the pattern of L-DOPA-stimulated DA release in the brain of 6-hydroxydopamine-lesioned rats, a rat model Parkinson's disease.

**Materials & Methods.** We used multi-site intracerebral microdialysis coupled to high performance liquid chromatography to simultaneously monitor DA extracellular levels in the prefrontal cortex (PFC), the hippocampus (HP), the striatum (STR) and the substantia nigra (SN) ipsilateral to the lesion. Rats received an acute intraperitoneal administration of L-DOPA preceded by the peripheral decarboxylase inhibitor benserazide (15 mg/kg).

**Results.** Acute L-DOPA (3-100 mg/kg) induced a diffuse and dose-dependent increase in DA release in all brain regions. The increase in DA release was regionally similar at the lowest dose of L-DOPA and 2-3 times stronger in the striatum at the highest dose. A lesion of 5-HT neurons using the intra-raphé administration of the 5-HT neurotoxin 5,7-dihydroxytryptamine, which lowered tissue 5-HT content and 5-hydroxyindole acetic acid (5-HIAA) by more than 90%, prevented L-DOPA-induced DA release in all brain regions. The administration of the NA neurotoxin DSP-4 (50 mg/kg), which lowered NA tissue content by 85%, enhanced L-DOPA-stimulated DA release in the hippocampus and the SNr. Blockade of 5-HT uptake sites using citalopram (4 mg/kg) reduced L-DOPA-stimulated DA release in all regions. Blockade of NA uptake sites (NET) using desipramine (10 mg/kg) or reboxetine (3 mg/kg) enhanced L-DOPA-stimulated DA extracellular levels in all regions (2-3 times) except the striatum. Chronic treatment with L-DOPA (12 mg/kg i.p. for 10 days) reduced basal 5-HT and 5-HIAA extracellular levels in all regions and reduced the ability of L-DOPA to release DA in a region-dependent manner.

**Conclusion.** The mechanism of action of L-DOPA on extracellular DA levels involves 5-HT fibres to release DA and NA fibers for clearance of extracellular DA. There are three main conclusions. The antiparkinsonian response to L-DOPA is conditioned by the functional status of 5-HT and NA neurons, themselves often altered in the disease. The mechanism of action implies a hypodopaminergy in the striatum and a hyperdopaminergy outside the striatum. The release of DA from 5-HT fibers should occur at the expense of correct 5-HT transmission.

# SEARCHING FOR ANTIPARKINSONIAN DRUGS CONSIDERING DOPAMINE AND ADENOSINE $A_{2A}$ RECEPTOR LIGANDS

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**Aim.** Recent studies provide an increasing evidence that adenosine $A_{2A}$ receptor ($A_{2A}$ AR) antagonists may be useful in treatment of Parkinson disease (PD) [1, 2]. Some of the performed experiments showed the benefits of $A_{2A}$ AR antagonists-including treatment, resulting in reduced dyskinesias associated with standard dopaminomimetic therapy and presenting additional neuroprotective effects [3]. These observations were further elucidated in Istradefylline testing in clinical trials [4]. The concepts for explanation of $A_{2A}$ AR antagonists utility in PD therapy take into account the role of $A_{2A}$ AR and dopamine receptor heterodimerization [5], involvement of both dopamine and adenosine receptors in GABAergic transmission [6] and interaction of $A_{2A}$ receptor signaling and the cholinergic system [7]. In our previous work we showed that selected adenosine $A_{2A}$ receptor antagonists possessed antiparkinsonian effects in "reserpine" and "oxotremorine" animal models [8]. These results persuaded us to develop novel compounds sharing structure elements of adenosine receptor ligands (xanthine) and dopamine fragment, which could act as dual target structures, with desired adenosine $A_{2A}$ AR antagonist and dopamine $D_2$ R agonist activity.

**Materials & Methods.** The series of 1,3-substituted purine-2,6-dione derivatives was tested in radioligand binding assays for their affinity towards adenosine receptors as described before [9]. The following receptor sources and radioligands were used: $A_1$ AR - rat brain cortical membranes, [$^3$H]CCPA; $A_{2A}$ AR - rat brain striatal membranes, [$^3$H] MSX-2. For 3 selected compounds antiparkinsonian activity was examined in animal model of haloperidol-induced catalepsy.

**Results.** Preliminary binding studies of novel hypothetical dual target compounds revealed that considered structures showed moderate adenosine $A_{2A}$ receptor affinity with $K_i$ values in micromolar/submicromolar concentration range. Moreover the most active $A_{2A}$ AR ligands represented good selectivity over $A_1$ subtype of adenosine receptors. Surprisingly in case of several structures a significant shift of selectivity towards $A_1$ AR was observed. One of tested compounds showed statistically significant effect in discussed antiparkinsonian test.

**Conclusions.** Basing on the promising results of the preliminary studies the next step should be the determination on intrinsic activity of considered compounds at $A_{2A}$ AR together with similar studies at dopamine $D_2$ receptors.

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# NOVEL AGONISTS FOR THE DOPAMINE $D_3$ RECEPTOR SUBTYPE WITH HIGH IN VIVO ACTIVITY

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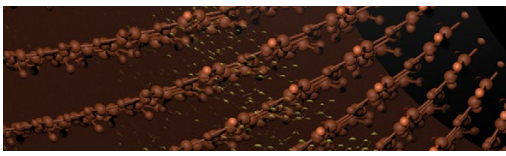
L-DOPA is still the gold standard treatment for motor functions with dopamine substitution therapy in patients with Parkinson´s disease. Dopamine receptor subtype agonists have great influence on therapeutic options as an ideal dopamine receptor agonist should fulfill the following criteria:
1) a physiological receptor profile with good anti-parkinson 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.



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# SALSOLINOL AFFECTS GASTROINTESTINAL MAST CELLS VIA LOCAL MECHANISMS

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**Background.** The catechol isoquinoline derivatives are endogenous compounds present in the mammalian brain and the representative one is referred to as salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). It may be formed by non-enzymatic or enzymatic pathway from aromatic amines precursors leading to neurotoxic N-methyltetrahydroquinolinium ions that may play a role in the etiology of Parkinson's disease. Three types of cells regulate the proper functions of the gastrointestinal tract: primary afferent neurons, enter-endocrine cells and immune cells. Each of these systems is more extensive than those of non-digestive organs. Mast cells may be regarded as prototypes of innate immune cells that can be controlled by neuronal mediators. Their activation has been implicated in many types of neuro-inflammatory responses, and related disturbances of gut motility, via direct or indirect mechanisms.

**Aim.** The aim of this study was to evaluate the influence of exogenous salsolinol on mast cells in the gastrointestinal tract of rats and on the histamine serum levels. Serum levels of salsolinol were measured as well.

**Material and Methods.** Male Wistar rats were subjected to continuous intraperitoneal dosing of salsolinol (200 mg/kg in total) with osmotic mini-pumps for two (S1 group, n=8) or four weeks (S2 group, n=8). An equivalent group of rats served as the control (C). At the end of the experiment animals were decapitated, blood and tissue samples were taken. Specimens from stomach, duodenum and proximal colon were toluidine blue stained and mast cells were counted in cross-sections. The total number and percentage of degranulated mast cells were assessed by image analysis. Serum samples were obtained by centrifugation and further analyzed by liquid chromatography–mass spectrometry. The limit of detection was set at 0.86 ng/ml for salsolinol and 2 pg/ml for histamine.

**Results.** The total number of mast cells in the gastrointestinal wall was decreased in both groups of salsolinol-treated rats compared to the control group. However the percentage of degranulated mast cells was elevated. The serum levels of histamine were significantly elevated in both groups of salsolinol-treated rats (especially in the S1 group) in comparison with the control group. Salsolinol was not detected in serum samples, which suggests the compound did not reach the systemic blood.

**Conclusions.** Our results suggest that salsolinol may directly activate mast cells in the gastrointestinal tract and thus, influence local neurotransmission. However, further research in the interplay between mast cells, immunological cells and the neuronal pathways is required.

# CHANGES IN THE EXPRESSION OF GALR1 AND GALR2 GALANIN RECEPTORS IN THE COLITIS CAUSED BY BRACHYSPIRA HYODYSENTERIAE INFECTION

***Krzysztof Wąsowicz, Michał Załęcki, Piotr Podlasz, Małgorzata Chmielewska, Katarzyna Łosiewicz, Jerzy Kaleczyc***

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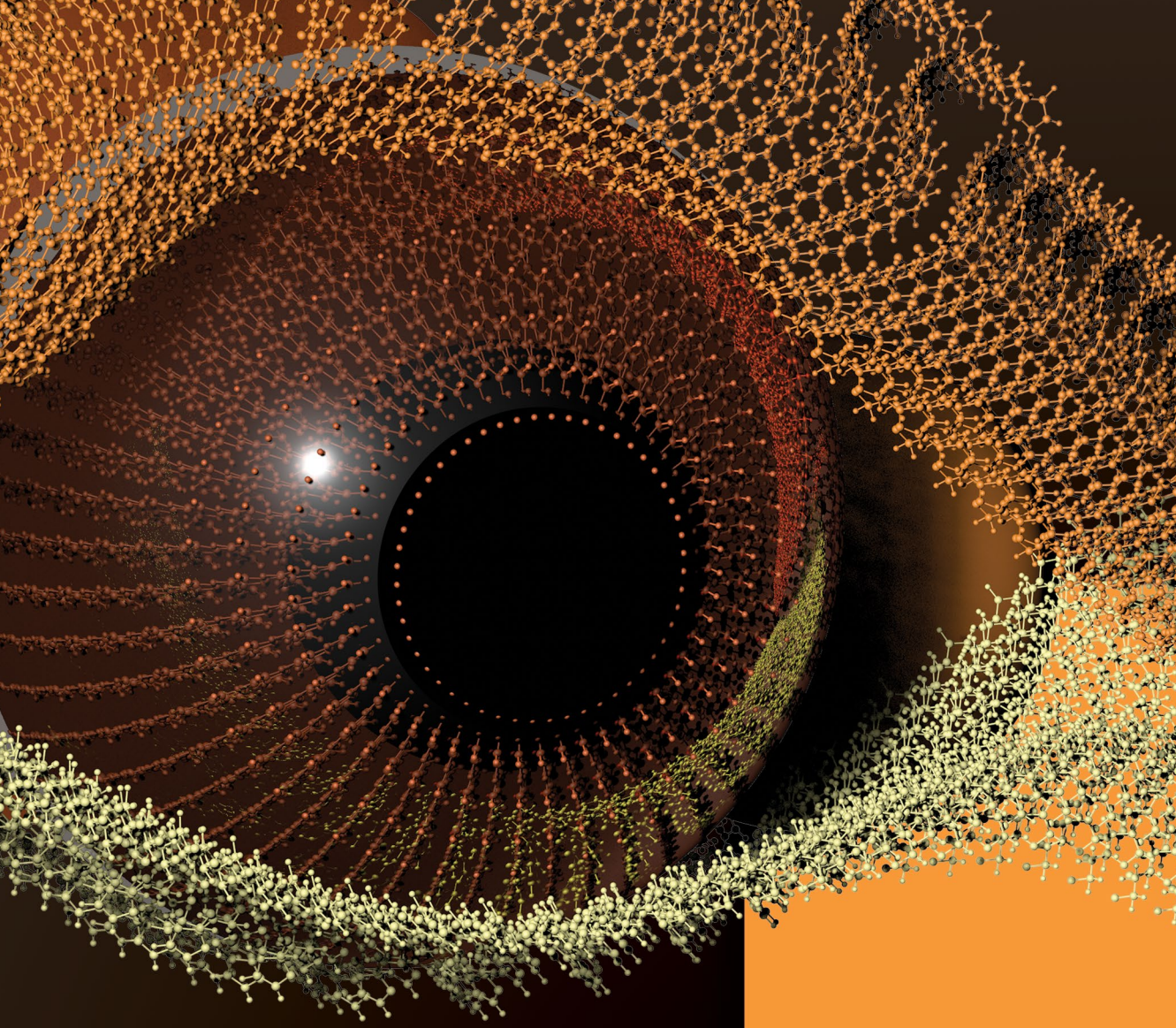
The expression of galanin (Gal) is raised in the porcine nervous system in many pathological processes (axotomy, neuronal degeneration, inflammation). The rise of Gal expression was found in the colon of pigs suffering from colitis caused by *Brachyspira hyodysenteriae*. The present study was aimed at the determination of changes in the expression of GalR1 and GalR2 galanin receptor in the muscular membrane, mucosa and lymphocytes isolated from mucosa in the *B. hyodysenteriae* infection.

Twelve gilts (body weight. ca 45 kg) of Large White Polish race were divided into control (n = 6) and experimental (n = 6) group. Experimental animals were infected orally (*via* an intragastric catheter) with a *B. hyodysenteriae* culture obtained from a State Veterinary Institute in Pulawy, Poland. After developing a severe haemorrhagic diarrhea the experimental animals were deeply anaesthetized with Thiopental (*i.v.*) and exsanguinated. The same procedure was used in control animals. After exsanguination the abdominal cavity was opened and the fragment of colonic wall from the centripetal turns was excised, washed in PBS and further processed. The fragment of the wall was separated into the muscular membrane and the mucosa and then the obtained fragments were preserved in the RNALater solution. From another part of the colonic wall sample the mucosa was scraped and used for lymphocytes isolation. Shortly, the mucosa was finely chopped, incubated in a dithiotreitol solution to get rid of the mucos and filtered through the Perlon wool. From the resulting cell suspension lymphocytes were isolated by the centrifugation in the Gradisol L (Polfa, Poland) gradient. The resulting lymphocyte pellet was also preserved in RNALater. Total RNA was isolated with a Total RNA mini kit (A&A Biotechnology, Poland) and the cDNA was prepared with a MMLV reverse transcriptase (Fermantas, Lithuania). Real Time PCR analysis was carried out using SYBR Green Master Mix (Roche, USA) and primers designed for porcine GalR1 and GalR2 receptor sequence and porcine GAPDH was used as an internal standard. The analysis was carried out in the ABI 7500 Fast Real-Time thermal cycler (Applied Biosystems, USA). The results were normalized against the GAPDH expression and statistically analysed with a GraphPad Prism 3.0 statistical package.

The results showed clearly a dramatic decrease in the level of GalR1 and GalR2 receptor mRNA. In the muscular membrane GalR2 dropped 5-fold (from 0.005 to 0.001, relatively to GAPDH), in the mucosa it dropped more than 30-fold (from 0.0066 to 0.0002), while in the isolated lymphocytes it dropped more than 20-fold (from 0.0442 to 0.002). Drops in the expression of GalR1 receptor were even more dramatic in the mucosa and lymphocytes. The differences were found to be statistically significant ($p<0.01$). The results point clearly to the very deep changes on GalR1 and GalR2 receptor expression in the colitis.



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