XII-th Conference of the Polish Histamine Research Society

XII Konferencja Polskiego Towarzystwa Badań nad Histaminą

Biogenic Amines and Related Biologically Active Compounds

Aminy Biogenne i Pokrewne Związki o Wysokiej Aktywności Biologicznej

Abstracts
Streszczenia wykładów i komunikatów





Dear Participants,

The Conference this year is very SPECIAL, as it is dedicated to Professor Bruno Mondovì from Rome, Italy, on the occasion of his eightieth birthday. I am proud and happy to be his friend for, at least, thirty years. I remember so clearly Professor Mondovi attending the European Histamine Research Society Meeting in Lodz in 1978, organised by Professor Maslinski and the staff of the Department of Biogenic Amines of the Polish Academy of Sciences, including myself, as it was just yesterday.
I am happy to host here, besides Poles, also colleagues/scientists from different European countries, namely: Ireland, Italy, Portugal and Spain, as well as from Japan, India and Nigeria.
Welcome to Lodz!
Welcome to the City with a long tradition of international cooperation and tolerance, which was for hundred twenty years (1820-1939) inhabited by people of various nationalities, with a dominance of the four: Poles, Germans, Jews and Russians. The foreigners used to come to develop textile industry, becoming permanent residents later on. Having such a differentiated national background, the Lodz inhabitants called themselves Lodzermenschen (or Lodzermen in English).
There is no more, or only a few, Lodzermen today, and the textile production ceased with 1991. Only the architecture of the City with richly decorated, luxurious mansions and redbrick factories has remained, being a proof of 19th/20 century industrial boom. I am sure; you will enjoy the beauty of art nouveau.
Today Lodz is a significant cultural and education centre and foreigners are coming for teaching, learning, attending scientific or other types of conferences, while many others, for touristic purposes.

Coming back to our Conference, I would like to thank all of you for your contributions, which deal with various aspects of the activity of biogenic compounds and their metabolism.
I believe, each of you will find something especially interesting to him or to her.
I wish the presenters and the audience much satisfaction from the lectures and lively discussions to follow, while all of us, I wish much satisfaction from knowledge and experience exchange. Let us enjoy being together again, both at the auditorium hall and at informal meetings.

Also, I wish you much joy from the performance at the Opera Theatre.

Finally, let me invite you to our next conference, in two years' time, also in Lodz.

W. Agnieszka Fogel
President of the Polish
Histamine Research Society

Conference programme

Hotel Ambasador, Lodz, Poland

Thurdsay, 23.10.2008

14:00 Arrival, accommodaction and registration, Hotel Ambasador, Kosynierów Gdyńskich 8 St., 93-320 Lodz

18:00 Opening Ceremony and Honorary Membership of Polish Histamine Research Society presentation to Prof. Bruno Mondovi (*Rome, Italy*) by Prof. W. Agnieszka Fogel, President of the Polish Histamine Research Society

19:00 Conference Lecture by B. Mondovì:
MODULATION OF AMINE OXIDASE ACTIVITY BY H_2O_2

20:00 Dinner

Friday, 24.10.2008

9:00-12:30 Session I, chaired by S. Maślinski and B. Skrzydło-Radomańska

Invited lecture by M. Unzeta:
PF9601N [N-(2-PROPYNYL)-2-(5-BENZYLOXY-INDOLYL) METHYLAMINE] A MAO B INHIBITOR, CONFERS NEUROPROTECTION IN MPP^+ AND ER STRESS-INDUCED DOPAMINERGIC CELL DEATH,
E. Sanz, J. L. Marco, M. Valoti, K. Tipton and M. Unzeta,
Institut de Neurociències & Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain; Instituto de Química Orgánica General, CSIC, Madrid, Spain; Department of Environmental Sciences "G. Sarfatti", University of Siena, Siena, Italy and Biochemistry Department, Trinity College, Dublin, Ireland

AMINE OXIDASES – SUBSTRATES AND FUNCTIONS,
K. F. Tipton, A. Olivieri, M. I. O'Sullivan and J. O'Sullivan,
Department of Biochemistry and Dublin Dental School and Hospital, Trinity College, Dublin, Ireland

SYNTHESIS AND BIOLOGICAL ASSESSMENT OF FUROQUINOLINES, PYRROLOQUINOLINES AND ANALOGUES TOWARDS CHOLINESTERASES AND MONOAMINE OXIDASES A AND B,
C. Martins, R. Léon, C. de los Ríos, M. C. Carreiras and J. Marco-Contelles,
iMED.UL, Faculty of Pharmacy, University of Lisbon, Portugal; ITH, Faculty of Medicine, Autonomous University, Madrid, Spain and Organic Chemistry Institute, Free Radical Lab, CSIC, Madrid, Spain

11:15-11:30 Coffee/Tea

BIOGENIC AMINE RECEPTORS SIGNALING PATHWAY CONNECTED WITH GQ PROTEINS IN PREGNANCY INDUCED HYPERTENSION,
A. Więcławek, U. Mazurek, H. Sławska, J. Szota and A. Oslislo,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland and Department of Gynaecology, Obstetrics and Oncological Gynaecology, Medical University of Silesia, Bytom, Poland

BIOACTIVE NATURETIC PEPTIDES AND HISTAMINE RELEASE IN PLACENTAL CIRCULATION,
D. Szukiewicz,
Department of General & Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

ADRENERGIC RECEPTORS SIGNALING PATHWAY CONNECTED WITH Gs/Gq PROTEINS IN PREGNANCY INDUCED HYPERTENSION,
A. Więcławek, U. Mazurek, J. Szota, H. Sławska and A. Oslislo,
Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland and Department of Gynaecology, Obstetrics and Oncological Gynaecology, Medical University of Silesia, Bytom, Poland

FRACTALKINE AFFECTS THE PERMEABILITY OF HUMAN AMNION TO CALCIUM IONS,
D. Szukiewicz, T. K. Mittal, M. Pyzlak and S. Maśliński,
Department of General & Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

13:00 Lunch

14:15-16:00 Session II, chaired by D. Maślińska and K. Walczyński

IN VIVO MODELS OF NEURODEGENERATIVE DISEASES: BINGE DRINKING,
L. Della Corte, D. T. Dexter, P. de Witte, F. Lallemand, F. Scholl, M. A. Colivicchi, C. Ballini and R. J. Ward,
Department of Preclinical and Clinical Pharmacology, University of Florence, Florence; Department of Cellular and Molecular Neuroscience, Imperial College, London UK, Italy and Biologie de Comportement, Université Catholique de Louvain, Belgium

RAT INTESTINAL PRECISION-CUT SLICES AS TOOL TO STUDY DRUG INTERACTIONS WITH TRANSPORT PROTEINS,
S. Dragoni, G. Materozzi, M. Frosini and M. Valoti,
Department of Biomedical Sciences, University of Siena, Siena, Italy

IS MELATONIN INVOLVED IN THE IRRITABLE BOWEL SYNDROME?
B. Skrzydło-Radomańska, P. Radwan, K. Radwan-Kwiatek and K. Laskowska,
Departament of Gastroenterology, Medical University of Lublin, Lublin, Poland

CHANGES IN CD2+, CD5+ AND CD21+ SUBPOPULATIONS OF LYMPHOCYTES AND CONCENTRATIONS OF SUBSTANCE P AND GALANIN IN ILEUM AND ILEAL LYMPH NODES IN THE COURSE OF SWINE DYSENTERY,
K. Wasowicz, A. Winnicka, W. Sienkiewicz, P. Podlasz, J. Kaleczyc, M. Lakomy,
Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland and Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

16:00-16:15 Coffee/Tea

16:15-17:30 Poster session, chaired by U. Mazurek and K. Wąsowicz

THE PROTECTIVE PROPERTIES OF THE ACETYLENIC TRYPTAMINE DERIVATIVE, PF9601N, AGAINST EXCITOTOXIC DAMAGE,
I. Bolea, C. Ballini, M. A. Colivicchi, M. Fattori, J. L. Marco, M. Unzeta and L. Della Corte, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain; Dip. Farmacologia Preclinica e Clinica, Università degli Study di Firenze, Florence, Italy and Inst. Quimica Organica General (CSIC), Madrid, Spain

ANTICONVULSANT PROPERTIES OF SOME HISTAMINE H_3 RECEPTOR LIGANDS,
K. Kieć-Kononowicz, M. Więcek, D. Łażewska, K. Kuder and H. Stark,
Jagiellonian University, Medical College, Faculty of Pharmacy, Department of Technology and Biotechnology of Drugs, Cracow, Poland and Institute of Pharmaceutical Chemistry, Johann Wolfgang Goethe-University, ZAFES/CMP, Frankfurt, Germany

ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF HISTAMINE H_3 RECEPTOR LIGANDS,
D. Łażewska, A. Więckowska, B. Malawska and K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs and Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Cracow, Poland

BETA-ADRENOCEPTOR-MEDIATED CYCLIC AMP SIGNAL IN DIFFERENT TYPES OF CULTURED CELLS: PHARMACOLOGICAL CHARACTERIZATION,
M. Berezińska, A. Wiktorowska-Owczarek and J. Z. Nowak,
Departament of Pharmacology, Chair of Pharmacology and Clinical Pharmacology, Medical University, Lodz, Poland

NEW 1-BENZYL-4-HYDROXYPIPERIDINE DERIVATIVES AS NON-IMIDAZOLE HISTAMINE H_3-ANTAGONISTS
I. Masłowska-Lipowicz, M. Figlus, O. P. Zuiderveld and K. Walczyński,
Department of Synthesis and Technology of Drugs, Medical University, Łódź, Poland and Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Vrije Universiteit, Amsterdam, The Netherlands

HYPOCRETIN INHIBITS CYCLIC AMP PRODUCTION IN PRIMARY NEURONAL CULTURES FROM RAT CEREBRAL CORTEX,
J. B. Zawilska, A. Woldan-Tambor and A. Urbańska,
Department of Pharmacodynamics Medical University of Lodz, Lodz, Poland and Institute for Medical Biology, Polish Academy of Sciences, Lodz, Poland

17:30-18:00 Sandwich/coffee/tea

18:20 Bus transfer to the City

19:00-21:20 Operetta by E. Calman “Graffin Marica” at Opera Theatre Lodz

21:30 Dinner, Orfeusz Restaurant
Transfer back

Saturday, 25.10. 2008

9:30-12:30 Session IV, chaired by M. Unzeta and J. Jochem

Invited lectures:

HISTAMINE PROMOTES MAST CELL GRANULE MATURATION IN AN AUTOCRINE MANNER,
S. Tanaka, Department of Immunobiology, School of Pharmaceutical Sciences, Mukogawa Women's University, Koshien, Nishinomiya

MAST CELL RESPONSE TO HYPOXIC CONDITIONS,
J. Dastych,
Institute for Medical Biology, Polish Academy of Sciences, Lodz, Poland

11:00-11:15 Coffee/Tea

INVOLVEMENT OF THE CHOLINERGIC SYSTEM IN THE CENTRAL HISTAMINE-INDUCED REVERSAL OF CRITICAL HAEMORRHAGIC HYPOTENSION IN RATS,
M. Yalcin, V. Savci and J. Jochem,
Department of Physiology, Faculty of Veterinary Medicine, Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine Uludag University, Bursa, Turkey and Department of Basic Medical Sciences, Faculty of Public Health, Medical University of Silesia, Bytom, Poland

OPIOID AND CANNABINOID SYSTEMS INVOLVEMENT IN MECHANISM CONTROLLING AN INCREASED ALCOHOL PREFERENCE IN PORTOCAVAL-SHUNTED RAT,
A. Stasiak and W. A. Fogel,
Department of Hormone Biochemistry, Medical University of Lodz, Lodz, Poland

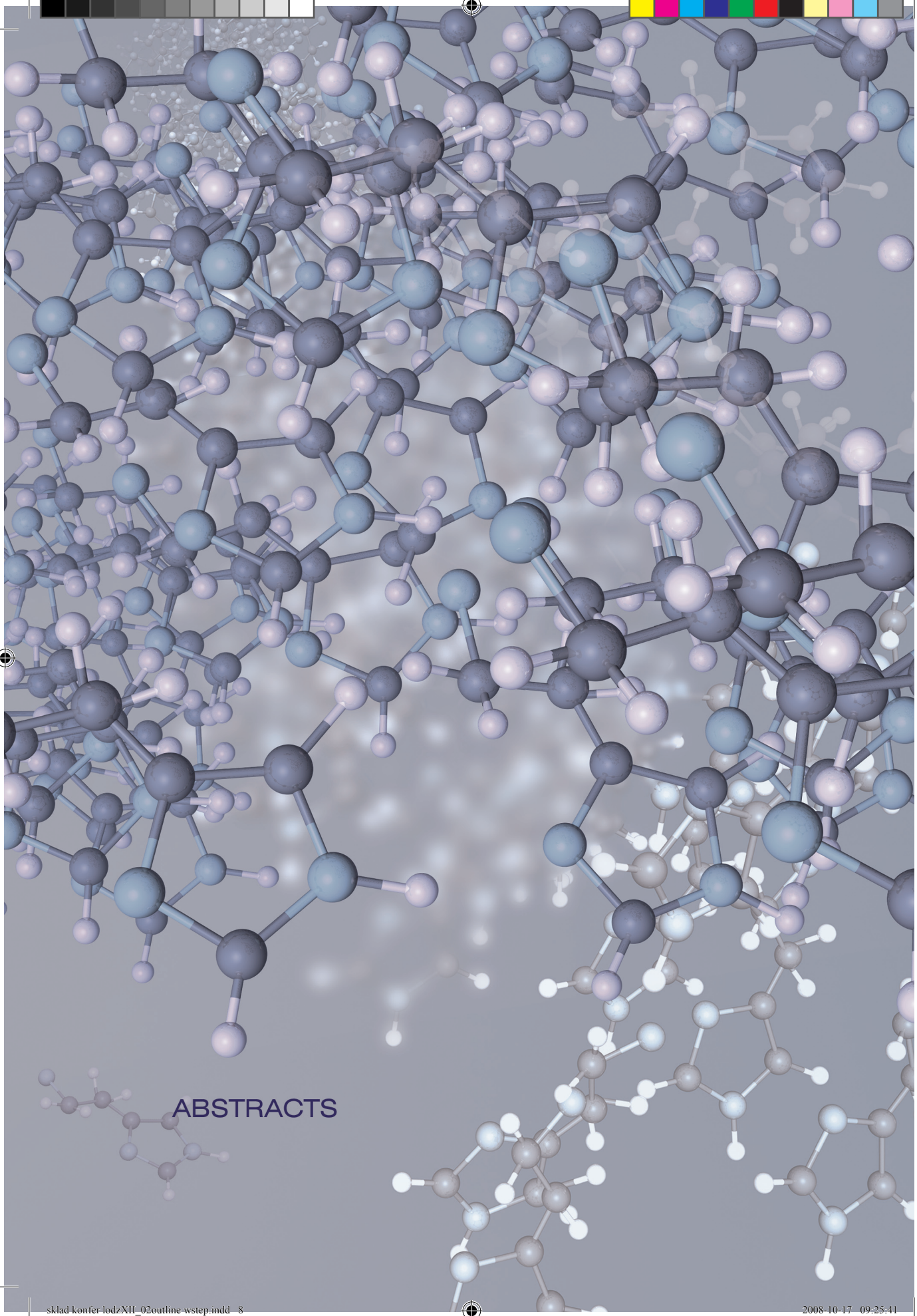
BIOGENIC AMINES – THE PRESENT AND THE FUTURE,
W. A. Fogel,
Department of Hormone Biochemistry, Medical University of Lodz, Lodz, Poland

12:00 Closing Ceremony of the XII-th Conference of the Polish Histamine Research Society

12:30 Lunch

14:00-17:00 COST D 34 Members one-to-one discussion

18:00 Cultural Event and Dinner



ABSTRACTS

MODULATION OF AMINE OXIDASE ACTIVITY BY H_2O_2

P. Pietrangeli, L. Morpurgo, B. Mondovì

Department of Biochemical Sciences "A. Rossi Fanelli" and C.N.R. Institute of Molecular Biology and Pathology, University of Rome "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy

A broad spectrum of possible functions of Cu-AOs, ranging from cell adhesion, cell growth regulation, to clinical applications are described. Their action may be related to the control of H_2O_2 levels as they are reported to be inactivated by the same H_2O_2 they produce, upon prolonged exposure to amine in excess over oxygen. This was demonstrated in pea seedling and pig kidney amine oxidase, and in bovine serum amine oxidase (BSAO). For the latter enzyme, inactivation by aldehydes, assisted by H_2O_2, was also reported. H_2O_2, which was formerly considered as an undesirable toxic product of oxygen reduction, is now believed to be involved in the regulation of cellular functions as intracellular messenger [1]. A condition for inactivation was the TPQ cofactor of BSAO to be in reduced form. Then, the TPQ reduced form was stabilized by inactivation, since a band appeared at 310 nm in the uv-vis difference spectrum with respect to native BSAO, while the reactivity with carbonyl reagents decreased. The Cu^{2+} EPR signal was not affected by inactivation, but, as reported above, a radical of low intensity formed at g = 2.001. The radical might derive from a conserved residue in proximity of the active site, such as the tyrosine at hydrogen-bonding distance of TPQ ionized hydroxyl, which becomes ionized upon TPQ reduction.

The proposed mechanism of inactivation was confirmed in *Lathyrus cicera* (red vetchling) amine oxidase (LCAO) [2], which, at difference from BSAO, forms the Cu^{+}-semiquinolamine radical, with a characteristic uv-vis spectrum, when oxygen is exhausted by an excess of amine in a closed cuvette. The inactivation, lasting about 90 min, is simultaneous with the radical decay and with the formation of a broad band (shoulder) at 350 nm. No inactivation occurs, when a thousand-fold excess of amine is rapidly oxidized in a LCAO solution stirred in open air. Thus, the inactivation is a slow reaction of the reduced enzyme with H_2O_2, following the turnover phase. Catalase protects LCAO from inactivation. This effect is substrate-dependent, varying from full protection (benzylamine) to no protection (putrescine). In absence of H_2O_2, a specific inactivating reaction, without formation of the 350 nm band, is induced by some aldehydes, notably putrescine.

The inactivation by H_2O_2 may be part of an auto-regulatory process in vivo, possibly relevant to cell adhesion and redox signalling. In this context the VAP-1 adhesion protein was in fact demonstrated to be a Cu-AO, with high homology with BSAO, but very low AO activity [3].

References:

1. Rhee, S. G. 1999. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp. Mol. Med.* 31:53-59.
2. Pietrangeli, P., Nocera, S., Federico R., Mondovì, B. and Morpurgo L. 2004. Inactivation of copper-containing amine oxidases by turnover products. *Eur J Biochem.* 271:146-152.
3. Salminen, T. A, Smith, D. J., Jalkanen, S. and Johnson, M. S. 1998. Structural model of the catalytic domain of an enzyme with cell adhesion activity: human vascular adhesion protein-1 (HVAP-1) D4 domain is an amine oxidase. *Protein Engng* 11:1195-1204.

PF9601N [N-(2-PROPYNYL)-2-(5-BENZYLOXY-INDOLYL) METHYLAMINE] A MAO B INHIBITOR, CONFERS NEUROPROTECTION IN MPP+ AND ER STRESS-INDUCED DOPAMINERGIC CELL DEATH

E. Sanz[1], J. L. Marco[2], M. Valoti[3], K. Tipton[4] and M. Unzeta[1]

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PF9601N [N-(2-propynyl) 2-(5-benzyloxyindol) methylamine] is a non-amphetamine type MAO-B inhibitor that has shown neuroprotective properties *in vivo* using different experimental models of Parkinson's disease. The mechanisms underlying its neuroprotective effects are poorly understood, but appear to be independent of MAO-B inhibition. We have studied its neuroprotective properties using the human SH-SY5Y dopaminergic cell line exposed to 1-methyl-4-phenylpyridinium (MPP+), a cellular model of Parkinson's disease. PF9601N pretreatment significantly reduced MPP+-induced cell death as well as the activation of one of the main executioner caspases, caspase-3. MPP+ induced stabilization of transcription factor p53, its nuclear translocation and transactivation of p53 response elements, whereas PF9601N prevented this increase, thus reducing its transcriptional activity. Additional results show that p53 may mediate its pro-apoptotic actions through caspase-2 under our experimental conditions. According to our data, PUMA-alpha may also contribute to the p53-induced cell death. Since PF9601N significantly reduced MPP+-induced caspase-2 activity and PUMA-alpha levels, this reduction may lead to increased cell survival.

On the other hand endoplasmic reticulum (ER) stress has recently been proposed as one of the factors contributing to apoptotic cell death in Parkinson's disease (PD). Therefore, we have studied the potential usefulness of PF9601N, in preventing cell death in a cell culture model of ER stress. Exposure to the ER stressor brefeldin A led to the activation of the unfolded protein response (UPR), as assessed by XBP1 splicing and eIF2-alpha phosphorylation, resulting in the expression of the URP-induced apoptotic mediator GADD153/CHOP. PF9601N pretreatment blocked brefeldin A activation of UPR as well as the caspase-2 and caspase-9 activation by this ER-stress toxin. In summary, our data suggests that PF9601N is able to block the responses elicited by ER stress. Thus, PF9601N is a novel molecule with different neuroprotective mechanisms, with a promising potential as a therapeutic agent in the treatment of neurodegenerative diseases.

AMINE OXIDASES – SUBSTRATES AND FUNCTIONS

K. F. Tipton[1], A. Olivieri[1], M. I. O'Sullivan[2], J. O'Sullivan[1,2]

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Monoamine oxidases (EC 1.4.3.4; MAO) and the, copper-containing, semicarbazide-sensitive amine oxidases (SSAO; formerly EC 1.4.3.6, but now reclassified as 1.4.3.21- primary-amine oxidase), have different tissue and cellular localizations but partially overlapping substrate specificities. Methylamine is a specific substrate for SSAO whereas secondary amines, such as adrenaline, are specific for MAO. Both enzymes catalyse oxidative deamination of amines to form hydrogen peroxide, an aldehyde plus ammonia or a substituted amine. The different cellular localizations mean that the H_2O_2 formed may have different messenger functions and potential toxicity under pathophysiological conditions. Conditions can be found where these amine oxidases can generate sufficient H_2O_2 to cause apoptosis in model systems but the relative importance of each of them and the possible significance in relation to human diseases remain to be established. Endothelial SSAO also functions as a vascular-adhesion protein (VAP-1) in the immune process. Studies with model compounds indicate that H_2O_2, generated during amine oxidation, is essential for the vascular adhesion process to occur. The nature of the amine substrate that drives this process is uncertain. We have shown that the anxiolytic and anti-allergic histamine H1-receptor antagonist hydroxyzine is also a selective reversible inhibitor of SSAO.

The H_2O_2, generated in both the MAO and SSAO catalysed reactions stimulates the recruitment of the GLUT 4 glucose transported to the cell surface in some tissues, thus increasing glucose uptake. We have produced evidence that adrenaline may be the physiological substrate for GLUT 4 recruitment. It is a substrate for MAO but not SSAO. However, oxidation of adrenaline by MAO releases both H_2O_2 and methylamine for further oxidation by SSAO. We have shown that the adrenaline stimulation of the GLUT 4-dependent increase in glucose uptake is abolished by MAO inhibition and reduced by SSAO inhibition. In contrast MAO inhibitors abolished the stimulating effects of noradrenaline with SSAO inhibition having no effect. The H_2O_2 formed in the SSAO-catalysed reaction also appears to play a role in cellular maturation and extracellular matrix formation but there is no evidence for the involvement of MAO in these processes, presumably reflecting the different localizations of these enzymes.

Dental pulp contains a form of SSAO that appears to be unique in its ability to oxidize serotonin. Immunological studies also indicate this enzyme to be associated with the pulpal nerves, whereas the enzyme is absent in nerves from other tissues, suggesting a tissue-specific function as a neurotransmitter-modulating enzyme within this tissue. The ability of SSAO in dental pulp to oxidize serotonin indicates that it may also have a tissue-specific role in responses to inflammation.

Acknowledgements:
We are grateful to Science Foundation, Ireland, the Health Research Board and COST action D34

SYNTHESIS AND BIOLOGICAL ASSESSMENT OF FUROQUINOLINES, PYRROLOQUINOLINES AND ANALOGUES TOWARDS CHOLINESTERASES AND MONOAMINE OXIDASES A AND B

C. Martins[1], R. Léon[2], C. de los Ríos[2], M. C. Carreiras[1], J. Marco-Contelles[3]

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Background. Alzheimer's disease (AD) is a neurodegenerative, irreversible disorder involving a progressive loss of cognitive functions and motor abilities, accompanied by behavioral disturbances that invariably lead to premature death. The hallmarks of the disease are the presence of senile plaques, neurofibrillary tangles and prominent cortical neuron loss. In addition, the levels of many neurotransmitters are greatly reduced. Depletion of acetylcholine (ACh) represents the most important event but other neurotransmitters such as serotonin, noradrenalin, dopamine, glutamate and substance P are also involved.
Cholinergic therapy for AD initially concentrated on acetylcholinesterase (AChE) inhibition since this is the main enzyme involved in the hydrolysis of ACh in the normal brain. However, ACh is also a substrate for butyrylcholinesterase (BuChE). Recent research has shown that in the cortex of patients affected by AD, AChE decreases progressively while BuChE activity is unchanged or even increased. With progression of the disease, both AChE and BuChE accumulate with amyloid β (Aβ) peptides intensifying Aβ neurotoxicity.
The multi-factorial nature of AD supports the most current therapeutic approach based on the multi-target strategy in drug design, which include drug candidates designed to act on multiple neural and biochemical targets for the treatment of cognition impairment, depression and neurodegeneration. Furoquinolines, pyrroloquinolines and analogues were designed to act on both the cholinergic and monoaminergic systems as well as calcium channel antagonists in order to display neuroprotective properties.

Methods. The syntheses were accomplished following the procedures described in the literature. The compounds were evaluated towards AChE and BuChE according to the Ellman`s protocol, using AChE from electric eel and acetylcholine chloride as a substrate for AChE assays and BuChE from horse and butyryl thiocholine chloride for the BuChE assays. Tacrine was used as the reference compound. Monoamine oxidase (MAO) inhibition assays were carried out with a fluorescence-based method using kynuramine as substrate.

Results. In the class of furoquinolines the most striking observation is their strong selectivity either towards AChE or BuChE. A few potent AChE inhibitors are also reported. In the pyrroloquinolines class the study also shows potent and selective BuChE inhibitors.
MAO studies concerning MAO-A and MAO-B are in course. In general, the tested compounds are moderate inhibitors of the MAO enzymes.

Conclusion. The selectivity either towards AChE or BuChE is very interesting since in AD, inappropriate cholinesterase activity increases the risk and progression of the disorder. In such instances, both AChE and BuChE may be appropriate targets and well-tolerated inhibitors of the both enzymes may have utility in the treatment of AD.
If the MAO inhibitory studies show a potent MAO-A or MAO-B inhibitor, docking studies will be performed in order to identify the binding mode and, maybe, suggest some chemical modulation to increase inhibition potency.

BIOGENIC AMINE RECEPTORS SIGNALING PATHWAY CONNECTED WITH G_Q PROTEINS IN PREGNANCY INDUCED HYPERTENSION

A. Więcławek[1], U. Mazurek[1], H. Sławska[2], J. Szota[1], A. Oslislo[2]

[1]Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland;
[2]Department of Gynaecology, Obstetrics and Oncological Gynaecology, Medical University of Silesia, Bytom, Poland

Background. Among metabotropic biogenic amine receptors, which interact with G_q proteins are: ADRA1 receptor (α–1 adrenergic), DRD2 receptor (dopaminergic D_2) and serotonin receptor HTR2B (5–HT_{2B}). Stimulated G_q proteins immediately activate phospholipase C. Phospholipase C hydrolyzes phosphatidylinositol bisphosphate (PIP2) to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 is able to open a calcium channel and release calcium ions stored inside the endoplasmic reticulum into the cytoplasm. Calcium through the medium of proteins such as: calmodulin and calcineurin influences many cellular processes, for example leads to contraction of smooth muscles of blood vessels. This phenomenon has an important role in pathogenesis of pregnancy induced hypertension (PIH).
The aim of this study is evaluation of transcription activity changes of biogenic amine receptors signaling pathway which interact with G_q proteins in placentas from pregnant women with PIH in comparison to the control group. Demonstration of significant changes in transcription activity may lead to more effective therapeutic strategies making use of suppressed or supplements gene therapy and, first of all, it will allow to gain an insight into PIH pathomechanism.

Methods. Placenta tissues collected from pregnant women with PIH (6 patients) and the control group (3 patients) during caesarean section were included in this study. Evaluation of transcription activity was performed by using Affymetrix HG–U 133A oligonucleotide microarrays. Statistical analysis of transcription activity results was performed using the SAM (*Significance Analysis of Microarrays*) program.

Results. Trancription activity analysis of biogenic amine receptors signaling pathway connected with G_q proteins showed, that in PIH statistically significant (Q=0%, Q is similar to p-value, adapted to the analysis of a large number of genes) increase of calmodulin and protein kinase C transcription activity in comparison to the control group was observed.

Conclusion. Transcription activity changes in biogenic amine receptors signaling pathway, which interact with G_q proteins and particularly calmodulin and protein kinase C transcription activity play an important role in pathomechanism of pregnancy induced hypertension.

BIOACTIVE NATRIURETIC PEPTIDES AND HISTAMINE RELEASE IN PLACENTAL CIRCULATION

D. Szukiewicz

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

Background. Absence of sympathetic innervation in placental vessels creates particular, humoral factors based regulation of vascular resistance within utero-placental circulation. In this circulatory system the members of bioactive natriuretic peptide family (c-type, atrial and brain natriuretic peptide; CNP, ANP and BNP, respectively) play an important role. Highest expression of CNP was found in the placenta, suggesting that CNP-related system may control the local vascular resistance as a vasodilatory endothelial component. Histamine released from vesicular structures of placental mast cells produces vasoconstriction in nitric oxide synthase inhibitor (NOLA)-containing perfusion fluid. Histaminergic H1 receptors are located on endothelial cells and promote release of vasodilators. Considering, that bioactive natriuretic peptides may induce degranulation of mast cells, the aim of the study was to investigate comparatively the influence of different doses of CNP, ANP and BNP on histamine release in placental fetal-side circulation.

Methods. Lobules (n=72) of term human placentas after normal-course pregnancies were perfused extracorporeally using Schneider's protocol. Perfusion fluid was isotonic and buffered (pH 7.4). Placental vessels were preconstricted by infusion of NOLA (100 µmol/l). CNP, ANP and BNP were infused (10 min increments at 2.5 ml/min) in the respective subgroups, using two concentrations: 50 nmol/l and 200 nmol/l. The control group was perfused with preconstriction only. Four specimens were obtained from each perfused lobule for histamine assay (fluorimetric method): 2 specimens before the start and 2 at the end of perfusion period (90 min). Perfusion pressure (PP) was monitored as an indicator of vascular reactivity. Pretreatment with H1 and H2 blockers (mepyramine or ranitidine) was introduced in respective subgroups. Statistical analyses were performed using the Mann-Whitney's test.

Results. Administration of the bioactive natriuretic peptides produced a dose-dependent transient decrease of PP. Significantly stronger ($p < 0.05$) vasodilation was observed for CNP (mean decrease of PP [kPa, ±SEM]: 1.19 ±0.11 and 2,63 ±0.13 for concentrations 50 nmol/l and 200 nmol/l, respectively), while the differences between ANP and BNP were not significant. The mean histamine level in placental specimens obtained after perfusion was significantly decreased ($p < 0.05$) for the higher concentrations of bioactive natriuretic peptides, compared to the control group. Interesingly, the biggest mean lowering of histamine level (before/after perfusion) was observed for CNP. H1 blockade reduced vasodilatory response the bioactive natriuretic peptides ($p < 0.05$), while the H2 blockade did not change this vascular reactivity.

Conclusion. The results indicate that CNP, ANP and BNP within placental circulation may produce local degranulation of the mast cells. Histamine released into placental vasculature may significantly modify vasodilatory effect of the bioactive natriuretic peptides, depending on its initial concentration, histamine H1 receptor status, activity of nitric oxide synthase and/or nitric oxide donors (e.g. L-arginine) availability. A link between cGMP-mediated endothelium-independent relaxation and endothelium-dependent pathway should be considered. Expecting that these not clearly understood dependencies are disturbed in some pathologic circulatory conditions involving placentae, further studies may be of a great potential from a clinical point of view.

ADRENERGIC RECEPTORS SIGNALING PATHWAY CONNECTED WITH G_s/G_Q PROTEINS IN PREGNANCY INDUCED HYPERTENSION

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Background. Adrenergic receptors are the members of metabotropic receptors, which biological effect depends on interaction with G proteins. ADRB receptors (β–adrenergic) cooperate with G_s proteins that stimulate adenylate cyclase activity, ADRA2 (α_2–adrenergic) receptors associate with G_i proteins and inhibit adenylate cyclase activity. Signaling pathway, which involves G_s/G_i proteins, regulates many cellular processes such as: increase of glycolysis, glycogenolysis, lipolysis, inhibition of glycogen biosynthesis and gluconeogenesis, moreover it leads to increase of sympathetic system activity.
The aim of this study is evaluation of transcription activity of the genes encoding proteins connected with adrenergic receptors signaling pathway which interact with G_s/G_i proteins in placentas from pregnant women with pregnancy induced hypertension (PIH) in comparison to a control group.

Methods. Transcription activity of 74 transcripts connected with adrenergic receptors signaling pathway which interact with G_s/G_i proteins in segments of placenta tissue received from a studied group of pregnant women with PIH (6 patients) and the control group (3 patients) during caesarean section was estimated by using HG–U 133A Affymetrix oligonucleotide microarrays. Statistical analysis was performed in the SAM (*Significance Analysis of Microarrays*) program.

Results. The obtained results of transcription activity analysis of adrenergic receptors signaling pathway connected with G_s/G_i proteins indicate, that mainly G_s signaling pathway was induced, simultaneously decrease of G_i transcription activity was observed. But these changes are not statistically significant. Only for A kinase anchor protein (AKAP) statistically significant, (Q=0%, Q is similar to p-value, adapted to the analysis of a large number of genes) decreased transcription activity in PIH in comparison to the control group was observed.

Conclusion. The received results of adrenergic receptors signaling pathway interacting with G_s/G_i proteins indicate, that in pregnancy induced hypertension changes of transcription activity of this pathway concentrate on AKAP protein, which is essential to protein kinase C activity.

FRACTALKINE AFFECTS THE PERMEABILITY OF HUMAN AMNION TO CALCIUM IONS

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Background. Chemokines are small proteins that stimulate the migration of leukocytes and mediate the course of an inflammatory reaction. Fractalkine (CX3CL1) is the only known member of the CX3C chemokine family. Amniotic endothelial cells produce fractalkine as a cell surface-bound as well as a cleaved soluble protein. In contrast to other chemokines, fractalkine displays potent chemoattractant activity for T cells, natural killer (NK) cells, and monocytes, but not neutrophils and is of non-haemopoietic origin. Chemokine receptors CX3CR1 were immunolocalized wihin the human fetal membranes Some reports suggest that various adverse conditions during pregnancy, including preterm uterine contractions and premature rupture of the membranes, are associated with increased fractalkine levels in amniotic fluid. This excess of CX3CL1 may lead to abnormal permeability of amnion to contractile factors. The aim of this pilot in vitro study was to examine the influence of fractalkine on permeability of human amnion to calcium ions (Ca^{++}).

Methods. Pieces of isolated extraplacental amnion (N=36) obtained after normal term pregnancies (N=12) were divided into 3 groups: fractalkine (10 µg/ml) treated, fractalkine + anti-CX3CR1 antibodies (1 µg/ml) treated and untreated controls (group I, II and III, respectively). Diffusion of Ca^{++} was measured at 37^0C in a system of plastic container divided into two 200 ml chambers by mounted amnion and filled with 0.9% saline. In all experiments the inner surface of the amnion (normally facing amniotic fluid) was mounted on the transmission chamber's side, while amniotic surface contiguous with the chorion was placed towards the receiving chamber. Exchange area was 4 cm^2. Initial concentration of Ca^{++} in saline of the transmission chamber was 6.0 mg%, resembling that observed in amniotic fluid. Saline mixing and circulation was assured by stirrers mounted in both chambers. Calcium concentration in the reception chamber was monitored every 2 h (1 ml samples collection) during the 12-hour experimental period. A colorimetric method based on complex formation with ortho-cresolphthalein was used. Calcium measurements were performed at wavelength of 570 nm. An inaccuracy in Ca^{++} measurements resulting from withdrawal of the samples (6 samples in all) was corrected using a mathematical model for the 2-compartment systems. Mann-Whitney's U-test were applied to check significance of observed differences in the mean Ca^{++} concentration between the three groups. The differences were deemed statistically significant if $p<0.05$.

Results. Fractalkine added to saline (group I) evoked up to 60% increase in amnion permeability for Ca^{++}, while simultaneous administration of anti-CX3CR1 antibodies (group II) reverted this effect even below the control levels (observed in group III; $p < 0.05$).

Conclusion. Increased permeability of human amnion to calcium ions due to fractalkine excess or CX3CR1 overexpression in amniotic epithelium may cause preterm, calcium-induced uterine contractions. However, the fact that we used saline instead of amniotic fluid produced some limitations on interpretation of these results.

IN VIVO MODELS OF NEURODEGENERATIVE DISEASES: BINGE DRINKING

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There is wide concern at the increasing occurrence of “binge drinking”, particularly in adolescence, which causes severe cognitive impairment in susceptible individuals. The pathogenesis of this lesion remains unclear but may be due to changes in immunological function, leading to microgliosis in specific brain regions.
Female rats (100 g at the commencement of the study) were subjected to a regime of “binge drinking” for 3 weeks, as previously described (Ward et al., 2008). Amino acids were then assayed in the CA1 hippocampal region, both before and after a further dose of ethanol, by microdialysis and HPLC (Bianchi et al., 1999). At the end of the experiment, phagocytic cells (alveolar macrophages) were isolated from each rat, and their ability to release inflammatory cytokines, before and after *in vitro* stimulation with lipopolysaccharide, assayed. Rat brains were removed, fixed in 4% paraformaldehyde and the activation of microglial cells within specific brain regions determined by OX6 staining.
Binge drinking regime, either 2g/kg or 3g/kg, enhanced the basal level of glutamate in the CA1 hippocampus, approximately 2 fold, although the last dose of ethanol did not affect the basal levels of glutamate, aspartate, taurine and GABA. The macrophages isolated from rats administered either a 2g/kg or 3g/kg ethanol binge drinking regime, exhibited a significant increase in iNOS prior to stimulation, as reflected by increased NO release. Furthermore, after stimulation there was a statistically significant (ANOVA, $P<0.05$) elevation of the LPS-stimulated NO release. A significant increase in microglial activation was observed also in the hippocampal regions of rats administered either binge regime of 2 or 3 g/kg ethanol.
Such results suggest that the pathogenesis of ‘binge-drinking’ may be mediated via the increased release of the excitotoxic neurotransmitter glutamate, resulting in the activation of microglia to release pro-inflammatory mediators. “Binge drinking” appears as a suitable model for neuroprotection studies in inflammatory-mediated conditions.

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Thanks are due for financial support of IREB to RJW, of ERAB to RJW, DTD and LDC, of ECRF and MPS Foundations to LDC and to EU COST D34 supporting our international cooperation.

RAT INTESTINAL PRECISION-CUT SLICES AS TOOL TO STUDY DRUG INTERACTIONS WITH TRANSPORT PROTEINS

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Intestinal metabolism (phase I and/or phase II) and drug transporters (phase III) have been recognized as major physiological mechanism to protect from toxic compounds and regulating their availability. Several phase III proteins commonly known as ATP-binding cassette (ABC) play key roles in tissue defense by transporting metabolic waste and toxic chemicals out of cells (Benet et al., 1999). To study xenobiotic interactions with MDR and MRP transporters intact cell systems are required. The aim of the present study was to set up intestinal precision-cut slice technique to study the interaction of ATP-dependent transporters with xenobiotics. Slices were prepared as described by De Kanter et al. (2005). Slices were individually incubated in RPMI 1640 under 95% O_2 5% CO_2 atmosphere at 37°C in 12 wells plates in presence of 0.5 μM calceinAM and various concentration of the well known MDR or MRP inhibitors verapamil, indomethacyn and glibenclamide. The intracellular deesterification of calceinAM in the fluorescent compound calcein was measured spectrofluorimetrically. The presence of transport inhibitors increased the intracellular concentration of calcein in time-dependent fashion and they showed the optimum incubation time at 30 minutes. Furthermore verapamil, indomethacyn and glibenclamide promoted a concentration-dependent accumulation of calcein (EC_{50} $3.28x10^{-6}$ M, $145x10^{-4}$ M, $190x10^{-6}$ M, respectively). This model was applied to study the interaction of new dihydropyridine derivatives. Among the tested compounds all showed to interact in dose-dependent manner with transporters present on the membrane of the enterocytes. This data suggests that the precision-cut intestinal slices could be a reliable, simple, and fast system to evaluate xenobiotic interactions with ABC transporters. Data present in literature indicates that this model is also suitable to study phase I and phase II drug metabolism (Van de Kerkof et al., 2007), suggesting that precision-cut slices gives the possibility to check phase I, II and III reaction, all involved in intestinal detoxifying mechanism.

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Keywords: Intestine, ABCtransporters, calcein

IS MELATONIN INVOLVED IN THE IRRITABLE BOWEL SYNDROME?

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There is a substantial evidence that large quantities of melatonin are produced in gastrointestinal tract, however, is still unclear which is the role of melatonin in digestive system in human physiology and pathophysiology. In the present study we investigated urinary excretion of a main melatonin metabolite, 6-sulphatoxymelatonin, in patients with irritable bowel syndrome (IBS). The investigation was carried out in 67 persons, both sexes, aged 20-45 years old who according to Rome III Criteria were diagnosed as sufferers of constipation (C-IBS, n=21 persons) or diarrhoea (D-IBS, n=24 persons) form of irritable bowel syndrome and as healthy subjects (K, n=22), matched for control. Samples were obtained from the collected diurnal urine. The concentration of 6-sulphatoxymelatonin level was measured with ELISA method, creatinine was automatically analyzed with biochemical analyzer and 6-SMLT/crea calculated. There were statistically significant differences between groups: the 6-SMLT/crea level was lower in C-IBS and D-IBS groups compared to K group. There were no differences between C-IBS and D-IBS groups, however there were observed differences between men and women with C-IBS. The 6-SMLT/crea level was higher in women with C-IBS compared to men with C-IBS. These results suggest that different melatonin secretion and metabolism may be involved in the pathogenesis of irritable bowel syndrome.

CHANGES IN CD2+, CD5+ AND CD21+ SUBPOPULATIONS OF LYMPHOCYTES AND CONCENTRATIONS OF SUBSTANCE P AND GALANIN IN ILEUM AND ILEAL LYMPH NODES IN THE COURSE OF SWINE DYSENTERY

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Background. The aim of the study was to examine changes in the number of CD2+, CD5+ and CD21+ lymphocytes as well as concentrations of Substance P (SP) and galanin (Gal) in the ileal lymphatic plate and ileal lymph nodes in the course of experimental infection with Brachyspira hyodysenteriae in pigs.

Methods. Eleven gilts aged 4 months were used in the study. Six clinically healthy animals were inoculated with a B. hyodysenteriae culture through a gastric catheter. 5 animals were left as controls. After the specific symptoms of the disease developed, all animals (infected and control) were deeply anaesthetized, exsanguinated and samples of the ileum with lymphatic plate and ileal lymph nodes were excised. For lymphocyte phenotyping the lymph nodes and ileum samples were finely chopped and vigorously shaken with PBS. The lymphocyte suspensions were incubated with specific primary antibodies for CD2, CD5 and CD21 antigens and secondary antibodies. The cells were analysed with FACScalibur and CellQuest. For determination of tissue concentrations of SP and Gal the tissues were homogenised and extracts were assayed with commercial ELISA kits. The results were read in a microplate reader.

Results. As regards the lymphocyte subpopulations, in ileal lymph nodes of control animals antigens CD2, CD5 and CD21 were expressed by 50.0, 63.7 and 52.7% of lymphocytes, respectively. In the ileum antigens CD2, CD5 and CD21 were expressed by 30.8, 55.0 and 62.2% of lymphocytes, respectively. In animals suffering from swine dysenteria statistically significant changes were regerding only CD21+ lymphocyte subpopulation. In lymph nodes of sick animals 25.8% of lymphocytes expressed antigen CD21, and in ileum the respective percentage was 43.9%. As regards the tissue concentration of Gal and SP, in the ileum of control animals concentrations (expressed per gram of wet tissue) of Gal and SP were 14.39 and 1.84 ng, respectively. In case of the lymph nodes the respective figures for Gal and SP were 12.04 ng and 5.5 ng. In animals suffering from swine dysenteria profound changes in tissue concentrations of the studied neuropeptides were detected. In the ileum of sick animals concentrations of Gal and SP were 23.35 ng and 3.03 ng, respectively. In case of the lymph nodes the respective figures for Gal and SP were 24.8 ng and 5.3 ng. All differences were found to be statistically significant.

Conclusion. The inflammation associated with swine dysentery affects tissue levels of SP and GAL, but as regards lymphocyte subpopulations only CD21+ lymphocytes were affected.

THE PROTECTIVE PROPERTIES OF THE ACETYLENIC TRYPTAMINE DERIVATIVE, PF9601N, AGAINST EXCITOTOXIC DAMAGE

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Introduction. Parkinson's disease (PD) is characterised by a progressive loss of the nigrostriatal dopaminergic neurons, leading to a severe depletion of dopamine in the substantia nigra and striatum. L-Deprenyl (Selegiline) is a classic MAO B inhibitor with antioxidant properties that has been used as an adjunct to levodopa therapy in the treatment of PD. PF9601N [N-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine], an acetylenic triptamine derivative, is a MAO B inhibitor, more potent and selective than l-deprenyl, that showed neuroprotective properties in several in vitro and in vivo models of PD (Perez & Unzeta, 2003) (Cutillas B, et al, 2002) (Sanz et al. 2004). Moreover, we have recently find out that the antiapoptotic effect of PF9601N are mediated by p53 pathway.

Objective. Excitotoxicity is a process involved in Parkinson's disease so the aim of the present work was to investigate the effects of PF9601N in an *in vivo* model of excitotoxicity.

Methods. We performed microdialysis experiments on male Wistar rats to investigate the effect of PF9601N pre-treatment on the kainate (KA) evoked release of the excitatory amino acids, glutamate and aspartate, and the amino acid taurine. We also made a preliminary immunohistological study, at 48h-post lesion, determining the glial activation and the presence of apoptotic nuclei.

Results. PF 9601N, administered i.p. (40 mg/kg), was able to reduce the KA-evoked release of glutamate and aspartate as well as increase taurine release. Regarding the histological study, we observed an astroglial and microglial activation as well as the presence of positive apoptotic nuclei provoked by kainate treatment. PF9601N pre-treatment prevented glial activation and significantly reduced the apoptotic cell death induced by kainate.

Conclusions. Our results show a protective effect of PF9601N against the excitotoxic lesion induced by kainate. Although further experiments must be done to elucidate the mechanisms of this action, these results allow us to suggest this new molecule as a good candidate for the treatment of Parkinson's disease and other neurodegenerative diseases that also involve excitotoxicity.

ANTICONVULSANT PROPERTIES OF SOME HISTAMINE H_3 RECEPTOR LIGANDS

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Background. Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures arising from excessive electrical activity in some portion of the brain, is a public health problem, which affects approximately 1% of the worldwide population.
Antiepileptic drugs (AEDs) can influence the inhibitory or excitatory neurotransmitter systems (GABA or glutamic and aspartic acid, respectively), or the ion transport across cell membranes. In the nineties it was demonstrated, that the central histaminergic neuronal system plays an important role in the inhibition of seizure activity. Some studies reported protection by H_3 receptor antagonists in different seizure models: in the maximal electroshock (MES) [1], kindling [2] and pentylenetetrazole induced convulsions [3].
In the present study the group of (homo)piperidine and piperazine ethers synthesized in accordance to general construction pattern of histamine H_3 receptor antagonists is presented. Their affinity to human histamine H_3 receptors (H_3R) was determined. Screening tests for anticonvulsant activity have now been performed.

Methods. Histamine H_3R affinities were evaluated in the binding assay at the human *h*H_3R. Displacement curves of [^{125}I]iodoproxyfan binding were measured at the *h*H_3R expressed in CHO-K1 cells stably transfected with the full-length coding sequence of the *h*H_3R [4-7].
Anticonvulsant properties of the obtained compounds were evaluated by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Health in Bethesda, USA. Preclinical phase of these screenings included two major convulsant tests: maximal electroshock (MES) and subcutaneous pentylenetetrazole (ScMet) as well as toxicity screen (Tox). The MES test is a model for generalized tonic-clonic (grand-mal) seizures, whereas the ScMet test is a model which primarily identifies compounds that raise seizure threshold. The neurological toxicity was evaluated in mice by rotorod test [8].

Results. The obtained compounds showed good to lack of affinity at human histamine H_3 receptors (K_i in the range from 19 to >1000 nM). Majority of them were active in the MES test in the dose 30 mg/kg 15 or 30 min after *i.p.* administration to mice and were inactive in the ScMet test. All compounds showed neurotoxicity in short time (30 min) after administration. A clear correlation between anticonvulsant activity and histamine H_3 receptor affinity could not been observed.

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ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF HISTAMINE H_3 RECEPTOR LIGANDS

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Background. Alzheimer's disease (AD), according to the cholinergic hypothesis, is caused by a decrease of cholinergic neurotransmission in the brain [1]. The only effective drugs for the symptomatic treatment of AD are acetylcholinesterase (AChE) inhibitors [2] and memantine, the NMDA-receptor antagonist. Although, AChE inhibitors promote memory function and delay the cognitive decline they have unpleasant side effects (gastrointestinal discomfort, risk of bradycardia). Histamine H_3 receptors (H_3Rs) are widely expressed in the mammalian brain. The blockade of H_3Rs increase brain histamine levels and the release of neurotransmitters (e.q. Ach, HA, DA) involved in cognitive processes [3]. The hybrid molecule acting as AChE inhibitor and histamine H_3R antagonist might be beneficial as anti AD drug increasing acetylcholine levels and reducing side effects of recent therapy.

Methods. AChE inhibitory activity was evaluated by spectrophotometrical Ellman's method using AChE from electric eel (2.5 units/1mL) [4]. The reaction took place in a final volume of 3.32 mL of 100 mM phosphate buffer, pH 8.0, containing 0.25 unit of AChE, 0.3 mM 5,5'-dithio- bis(2-nitrobenzoic) acid (DTNB, Ellman's reagent) and 0.45 mM acetylthiocholine as substrate.

Results. Tested compounds showed inhibitory action aganist AChE in micromol level (IC_{50} < 40 μM). The obtained results were compared with determined human histamine H_3R potency [5]. It was possible to identify compound 1-[3-(3-(4-chlorophenoxy)propoxy)propyl]-azepane combining relatively high inhibitory action on AChE (IC_{50}: 7.91 μM) and very high histamine H_3R potency (hK_i: 3.48 nM).

Conclusion. Investigations and synthesis of diether derivatives of homopiperidine should be performed to find further potent dual-acting compounds (AChE inhibitors-histamine H_3R antagonists).

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BETA-ADRENOCEPTOR-MEDIATED CYCLIC AMP SIGNAL IN DIFFERENT TYPES OF CULTURED CELLS: PHARMACOLOGICAL CHARACTERIZATION

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Endogenous catecholamines, such as dopamine (DA), noradrenaline (NA) and adrenaline (ADR), play a role of neurotransmitter (DA, NA) or a hormone (ADR). They all signal through different types of G-protein-coupled receptors, DA via specific DA receptors, whereas NA and A via common adrenergic receptors that are divided into two subfamilies: α-adrenergic receptors and β-adrenergic receptors; both subfamilies embrace receptor subtypes: α_1 and α_2, and β_1, β_2, or β_3. Of the mentioned adrenergic receptors, β-adrenoceptors are positively coupled to adenylyl cyclase (AC)/cyclic AMP (cAMP) signaling system; these receptors are targets for a great number of therapeutics. NA and A exert profound two-branched physiological effects within the central nervous system, and through the peripheral autonomic nervous system, the latter controlling or contributing to the control of the functioning of nearly all of the major organ systems of the body, including heart function, dynamics of blood vessels, or energy metabolism. Although the family of β-adrenoceptors is classically linked to cAMP signaling, recent evidence suggests that other (than AC/cAMP) intracellular signaling systems may be activated by stimulation of these receptors, and β_3-adrenoceptor-mediated generation of nitric oxide (NO) in endothelial cells is one of examples. Considering both physiological and therapeutic importance of β-family receptors, it is crucial to recognize cell/tissue localization of particular β-receptor subtypes, as well as their coupling with intracellular signaling and effector system/s.

This work is focused on NA/ADR-sensitive β-adrenoceptors linked with AC/cAMP signaling system in two types of cells, brain glial cells (astrocytes) which are targets mainly for the neurotransmitter NA and endothelial cells which represent the inner cellular lining of blood vessels and which are targets mainly for the hormone ADR, as well as astrocyte-derived tumor cells, i.e. C6 glial cells, which may be affected by both NA and ADR; the importance of these two signals for tumorigenesis remains unknown. Pharmacological characterization of cAMP signal in the three types of cultured cells (primary rat astrocyte cultures, rat C6 glial cells, human microvascular endothelial cells – HMEC-1) was carried out with the aid of an array of receptor active drugs, including agonists: adrenoceptor-nonselective (NA, ADR), α-adrenoceptor-selective (phenylephrine - PHE, methoxamine - METHOX, clonidine, CLO), β-adrenoceptor-selective (isoprenaline, ISO), and antagonists: α_1-selective (prazosin – PRAZ), α_2-selective (yohimbine – YOH), β-nonselective (propranolol – PROP), and β-subtype-selective (CGP20712 – β_1, ICI118551 – β_2, and SR59230A – β_3).

The obtained preliminary results are: 1. The agonistic profile of cAMP effect in astrocytes and C6 glial cells was similar: ISO ≥ NA > ADR >>> PHE ≈ METHOX), and differed from the drug profile seen in HMEC-1 cells: ADR ≈ ISO >> NA >> PHE > METHOX. 2. PROPR antagonized nearly completely the effect of ISO and only partially the effect of NA in astrocytes, and completely antagonized the effects of ADR and ISO in HMEC-1 cells. 3. Selective β_1-, β_2- and β_3-blockers revealed that in astrocytes and C6 glial cells the ISO-evoked cAMP effect was mediated mainly (or even exclusively) by β_1-adrenoceptor, whereas in HMEC-1 cells the effect of ADR was possibly mediated in 50% by β_2-adrenoceptor, however all three β-subtype specific drugs used together did antagonize the hormone effect to roughly the same extent, suggesting that HMEC-1 cells may possess β-adrenoceptor that does not fit the β_1-, β_2-, β_3-classification.

This study was supported by funds from Medical University in Lodz (grants: 502-11-580, 502-11-579, 502-11-462).

NEW 1-BENZYL-4-HYDROXYPIPERIDINE DERIVATIVES AS NON-IMIDAZOLE HISTAMINE H_3-ANTAGONISTS

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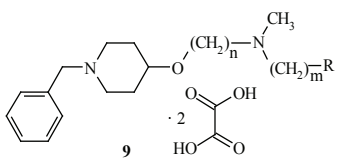
Background. During the years following number of H_3 antagonists belonging to different chemical classes, subsequently divided between classical imidazole-based and non-imidazole series have been described; the imidazole derivatives were considered to be less attractive for pharmacokinetic as well as for toxicological reasons. Orignally it was assumed that the imidazole ring was essential for H_3 receptor active ligands. As imidazole-derived ligands have an inhibitory effect of cytochrome P450 enzymes, caused by imidazole nitrogen complexation to heme iron in the active site of the enzyme and leading to drug-drug interactions and moreover show pharmacokinetic constraints. The development of potent non-imidazole H_3 receptor compounds became attractive for the search for potential medicines.
In the present work, we report the synthesis and preliminary pharmacological investigation of a new series of 1-benzyl-4-hydroxypiperidine-based H_3 histamine receptor antagonists **9** in which two concepts - rigidification of aminopropyloxy link by incorporation it into 4-hydroxypiperidine ring and replacement of phenyl (present in aminopropyloxyphenyl archetypal H_3 pharmacophore) by aminoalkyl and aminophenylalkyl chain.



9

where
n=3; 5
m=0; 2; 4 for R=CH_3 or n=1; 3; 5 for R=C_6H_5

Methods. A series of 1-benzyl-4-(3-aminopropyloxy)piperidine and 1-benzyl-4-(5-aminopentyloxy)piperidine derivatives has been prepared. The 1-benzyl-4-hydroxypiperidine derivatives obtained were evaluated for their affinities at recombinant human histamine H_3 receptor, stably expressed in HEK 293T cells. The most potent antagonists in this series were also in vitro tested as H_3 receptor antagonists - the electrically evoked contraction of the guinea-pig jejunum. Additionally, the histaminergic H_1 antagonism of selected compounds was established on the isolated guinea-pig ileum by conventional methods; the pA_2 values were compared with the potency of pyrilamine.

Results and Conclusions.
The 1-benzyl-4-[5-(*N*-methyl-*N*-subsitutedaminopentyloxy)]piperidines display a higher potency than their *N*-methyl-*N*-propyloxy analogues. The highest potency for both homologous series is seen in the compound with the *N*-methyl-*N*-propylaminopentyloxy substituent (pK_i=7.09) and with slightly lower potencies for compounds carrying on *N*-methyl-*N*-phenylpropylaminopentyloxy- and *N*-methyl-*N*-phenylpentylaminopentyloxy-substituent, respectively. Selected compounds were also tested for H_1 antagonistic effects *in vitro*, following standard methods, using the guinea pig ileum. They did not show any H_1-antagonistic activity.

HYPOCRETIN INHIBITS CYCLIC AMP PRODUCTION IN PRIMARY NEURONAL CULTURES FROM RAT CEREBRAL CORTEX

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Background. Hypocretins (also called orexins) are two newly discovered neuropeptides synthesized in the hypothalamus Their amino-acid sequences are highly conserved among vertebrates. Hypocretins act on their targets via two specific, membrane-bound, G protein-coupled receptors, Hcrtr-1 and Hcrtr-2. Among various physiological actions ascribed to hypocretins, the strongest evidence is for their involvement in the integration and stabilization of an arousal network. In contrast to numerous data on anatomy of hypocretin-containing neurons and the physiological actions of the peptides, relatively little is known about signal transduction pathways triggered by activation of their receptors. The aim of this study was to analyze effects of hypocretin-1 (the peptide exhibiting high affinity for Hcrtr-1 and Hcrtr-2 receptors) on basal and stimulated cyclic AMP formation in primary neuronal cultures from rat cerebral cortex.
Methods. Primary neuronal cell cultures were obtained from Wistar rat embryos on day 16 of gestation. The cultures were cultivated for 7 days prior to experimentation. Cyclic AMP formation and effects of drugs upon it was studied in [^{3}H]adenine prelabeled neuronal cultures. All experiments were carried out in the presence of 3-isobutyl-1-methylxanthine (100 μM), an inhibitor of cyclic nucleotides phosphodiesterase.
Results. Hypocretin-1 (0.001-1 μM) did not affect the basal cyclic AMP formation in primary neuronal cultures from rat cerebral cortex. Forskolin, a direct activator of adenylyl cyclase, used at concentrations of 1 and 3 μM increased cyclic AMP production to 180 and 330% control value, respectively. Hypocretin-1 (0.01-1 μM) inhibited, in a concentration-dependent manner, the forskolin-induced cyclic AMP accumulation. Incubation of primary neuronal cell cultures with 0.1 μM of pituitary adenylate cyclase-activating polypeptide (PACAP27) or 3 μM of vasoactive intestinal peptide (VIP) resulted in approximately 7-fold increase in cyclic AMP formation. This stimulatory effects of PACAP27 and VIP was inhibited in a concentration-dependent manner by hypocretin-1 (0.01-1 μM).
Conclusion. The obtained results suggest 1. that hypocretin receptors present in rat cortical neurons are coupled to receptors, whose activation leads to inhibition of cyclic AMP generation, and 2. the existence of a neurone-linked functional interaction between hypocretins and neuropeptides PACAP/VIP in rat cerebral cortex.

Supported by the Grant No 502-13-770 from the Medical University of Lodz.

HISTAMINE PROMOTES MAST CELL GRANULE MATURATION IN AN AUTOCRINE MANNER

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Background. We previously generated the gene-targeted mice that lack the functional gene encoding histidine decarboxylase (HDC). The $HDC^{-/-}$ mice were found to lack the cutaneous and systemic anaphylactic responses [1]. In addition, electric microscopic analyses revealed that cutaneous and peritoneal mast cells in the $HDC^{-/-}$ mice possess the aberrant granules with very low electric densities [2]. These findings raised a possibility that histamine is involved in granule maturation of tissue mast cells in addition to its roles as a potent pro-inflammatory mediator.

Methods and Results. No significant phenotypic differences were found in IL-3 dependent primary cultured mast cells (BMMCs) derived from bone marrow cells of between the wild type and $HDC^{-/-}$ mice. However, granule maturation judged by Safranin staining was severely impaired in $HDC^{-/-}$ cultured mast cells when they were co-cultured with Swiss 3T3 fibroblasts in the presence of stem cell factor. Granule maturation of the co-cultured $HDC^{-/-}$ mast cells was restored by adding histamine or clobenpropit, but not by dimaprit. Treatment with tetrabenazine, which is known as a potent inhibitor for VMAT-2, was found to abolish granule accumulation of histamine during the co-cultured period, but did not affect the granule maturation, indicating that granule storage of histamine is not required for granule maturation. RT-PCR analyses revealed that BMMCs express H_1, H_2, and H_4 receptor. A drastic increase in the cytosolic Ca^{2+} concentration was induced in BMMCs by histamine, which was suppressed by pretreatment with JNJ7777120, not with pyrilamine, indicating the expression of functional H4 receptor.

Conclusion. These results suggested that histamine promotes granule maturation in an autocrine manner during the process of maturation toward the connective tissue type mast cells. Although the detailed mechanism of histamine remains to be clarified, it is possible that the H4 receptor is involved in histamine-mediated granule maturation of mast cells.

References:

1. *Ohtsu, H. et al. Eur. J. Immunol. 32: 1698-1708, 2002*
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MAST CELL RESPONSE TO HYPOXIC CONDITIONS

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Mast cells play important role in many pathological conditions including asthma, rheumatic diseases and certain types of cancer where local hypoxia in tissues is observed. We investigated regulation of expression of hypoxia inducible factor-1α (HIF-1α), the major nuclear transcription factor governing changes in gene expression in response to hypoxic conditions, in human mast cells.

We observed that mast cell activators ionomycin and substance P increased amount of HIF-1α protein in mast cells activated under hypoxic conditions to the level greatly exceeding that observed under such conditions in resting mast cells. This upregulation of HIF-1α was sensitive to transcription inhibitor actinomycin-D (ActD) and inhibitors of calcineurin (CaN): Cyclosporin A (CsA) and FK506. Activation of mast cells with ionomycin and substance P has also resulted in several folds increase in HIF-1α mRNA and this increase was similarly to upregulation of HIF-1α sensitive to ActD and CsA. Analysis of HIF-1α promoter activity in ionomycin activated mast cells showed significant increase that was inhibited with CsA and FK506. *In situ* mutagenesis experiments showed that ionomycin-mediated HIF-1α promoter activity depends in significant part on conservative NFAT binding site. Thus HIF-1α accumulated in activated mast cells in a process that involves transcriptional upregulation of HIF-1α gene that depends on Calcineurin/NFAT signaling pathway and NFAT binding site in the HIF-1α promoter.

INVOLVEMENT OF THE CHOLINERGIC SYSTEM IN THE CENTRAL HISTAMINE-INDUCED REVERSAL OF CRITICAL HAEMORRHAGIC HYPOTENSION IN RATS

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Histamine, acting centrally as a neurotransmitter, evokes a reversal of haemorrhagic shock in rats due to the activation of the sympathetic and the renin-angiotensin systems as well as the release of arginine vasopressin and proopiomelanocortin-derived peptides. In the present study, we demonstrate influences of cholinergic receptor antagonists on the central histamine-induced resuscitating action. Experiments were carried out in male anaesthetised Wistar rats subjected to a haemorrhagic hypotension of 20-25 mmHg, resulting in the death of all control animals within 30 min. Histamine (100 nmol) administered intracerebroventricularly (icv) at 5 min of critical hypotension produced a long-lasting pressor effect with increases in heart rate and peripheral blood flows, and a 100% survival at 2 h. The effects were almost completely blocked by nicotinic receptor antagonist mecamylamine (246.3 nmol; icv) and partially inhibited by muscarinic receptor blocker atropine sulphate (14.8 nmol; icv). Cholinergic receptor antagonists given alone in the control saline-treated groups did not affect cardiovascular parameters in the post-bleeding period. In conclusion, there are interactions between the histaminergic and cholinergic systems, with an involvement of both nicotinic and muscarinic receptors, in the central cardiovascular regulation in haemorrhagic hypotension in rats.

OPIOID AND CANNABINOID SYSTEMS INVOLVEMENT IN THE MECHANISMS CONTROLLING INCREASED ALCOHOL PREFERENCE IN PORTOCAVAL-SHUNTED RATS

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Background: The liver atrophy, associated with portocaval anastomosis (PCA), results in different neurobiological and behavioural alterations. It has been suggested that PCA rat may be a useful model for studying the mechanisms, controlling ethanol preference, as PCA rat consumes voluntarily more alcohol than normal rat. Seeking the mechanisms, which underlie this phenomenon, a possible partaking of cannabinoid and opioid systems was investigated. *In vivo*, the influence of Rimonabant (CB1 receptor antagonist) on alcohol intake was examined in a free choice test paradigm. *Ex vivo* receptor binding studies, regarding brain delta opioid receptors, were also performed.

Methods: The free choice tests between 10% alcohol and tap water were performed before and after Rimonabant therapy. PCA rats (adult male Lewis, 6-8 months after operations), selected for the study, consumed voluntarily twice more alcohol than the sham-operated animals and suffered from liver insufficiency. Post-mortem examination confirmed that their liver weight/body weight ratio was significantly lower (1.80 ± 0.18 vs. 2.65 ± 0.18). Rimonabant was administered i.g. for 10 consecutive days, at the beginning of the dark phase of a 24 h cycle, in three different doses: 1.5, 3.0 and 10.0 mg per kg of body mass. During the drug administration the rats were kept individually in metabolic cages with a free access to water, alcohol solution and food. Feed and fluid consumption, as well as urine outputs, were recorded daily. The delta opioid receptor ligand – [3H]-DPDPE, Enkephalin, (2-D-penicillamine, 5-D-penicillamine), [Tyrosyl-2,6-3H(N)], was used to obtain binding characteristics of the PCA and control rat brain membranes.

Results: Alcohol consumption in PCA rats was not affected by Rimonabant therapy. The binding data suggest a higher density of brain delta opioid receptors and lower affinity of the radioligand to these receptors in PCA rats, as compared to control animals.

Conclusions: The lack of alcohol intake modulation by Rimonabant therapy does not exclude contribution of the cannabinoid system in the maintenance of exaggerated drinking; further studies are needed, including endogenic cannabinoids and receptors.
The findings, regarding delta opioid receptors, together with previous reports, suggest that increased alcohol preference in PCA rats may, in part, result from disturbances in the brain opioid system.

Acknowledgements: This study was supported by 502-17-520 and 502-17-520 grants from the Medical University in Lodz. Rimonababt was obtained from the USA NIMH's Chemical Synthesis and Drug Supply Program.

BIOGENIC AMINES – THE PRESENT AND THE FUTURE

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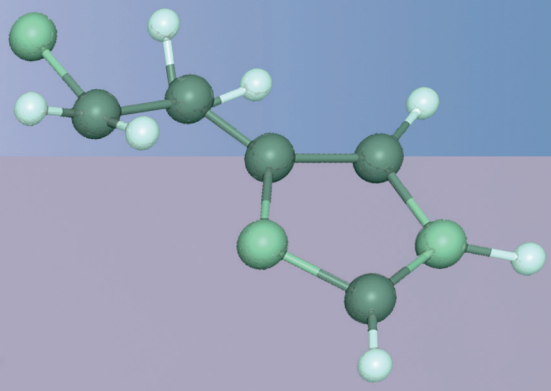
Biogenic amines are important components of mammalian organisms, expressing high biological activity. Constituting a uniform, informatic and regulatory system, classified as the local hormone group, they regulate virtually all running processes; these in a single cell, at the level of organs and of the whole body. Amines transmit external and internal signals, trigger responses; they contract or relax vessels and muscles, stimulate or inhibit secretory activity, influence the mood; support life and reproduction. Due to the modern technical approaches and new detection methods, the knowledge on the ubiquitous presence of the amine system, the network functioning in a specific manner, has been getting wider and deeper and better and better is also the understanding of the regulation of amine concentration, sensing proteins, metabolising enzymes and other system elements in a given organ.

At present, the therapy of many diseases, such as allergy, vascular hypertension, cardiovascular diseases, a variety of nervous system dysfunctions -from depression to neurodegeneration, involve a considerable use of the drugs, targeting the biogenic amine system, e.g., the blockers of amine receptors, inhibitors of their catabolizing enzyme activities, the blockers of proteins, involved in amine transport into or out of the cell, and amine antimetabolites.

The lecture reviews the current knowledge, concerning amine metabolism and functional significance of their enzymes, receptors and transporters, both in health and disease. Based on literature reports, it discusses the implementation of the most recent results of basic research into new therapeutical strategies and points to possible directions of future studies on biogenic amine systems.



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COST D34 WG 0003-05

Supported by
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