XIII Conference

XIII Konferencja

Biogenic Amines and Related Biologically Active Compounds

Aminy biogenne i pokrewne związki o wysokiej aktywności biologicznej

Lodz, Poland
2010
21-23 October

Joint Meeting
of the Polish Histamine Research Society & COST Action BM0806
"Recent advances in histamine receptor H_4R research"

fot.: M. Kawczyński.

Dear Participants,

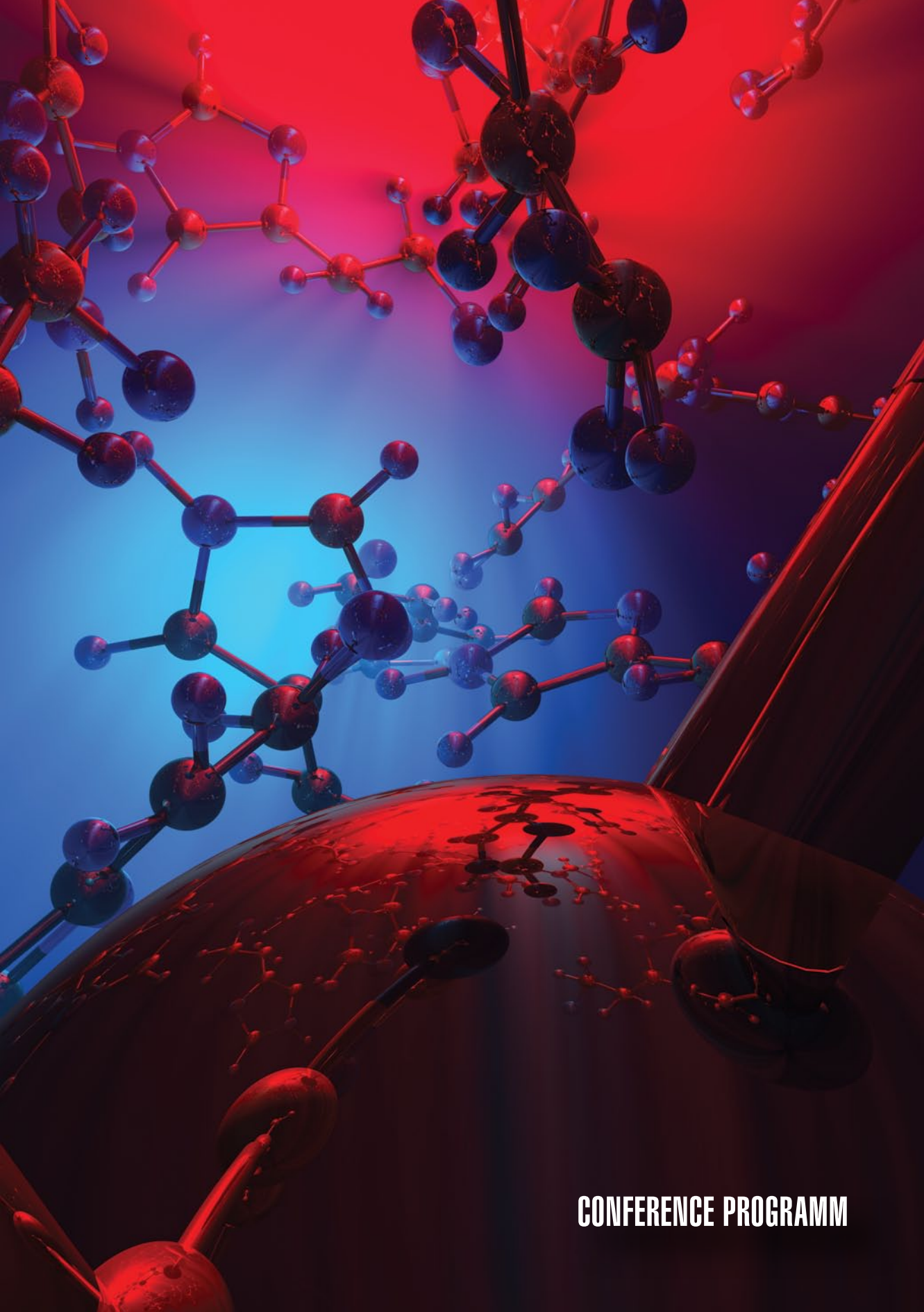
Welcome to our biennial Conference, devoted to a family of biogenic amines and their relatives with a high biological activity. This fascinating puzzle, consisting of amines and polyamines, their metabolites, enzymes, receptors, carriers, cytokines and so on, attracts still much attention. Nothing to add, most of the pharmaceuticals present today on the market are directed against any of component of biogenic amine system. And the deeper is the insight into the system, it becomes more obvious how complicated interrelations and interplays occur among the parties. Thus, recently, after the era of selective compounds that have replaced the earlier so-called "dirty drugs" (unpredicted targets), we have entered the period of synthesis and experimental evaluation of multitarget compounds. Some relevant reports will be presented during our Meeting.

Thank you very much for your coming, I am so glad you are here.
This time, the Polish Histamine Research Society share the pleasure and duty of conference organisation with COST Action BM0806, focused on histamine H4 receptors, and with the Department of Hormone Biochemistry, Medical University of Lodz, Poland.
As you see, the present conference bears the 13th number. According to statistics, roughly one per ten people is superstitious and, most commonly it is the Europeans and Americans who fear number 13, while for example, such a number for the Asians is 4. The triskaidekaphobia or tetraphobia leads some people to avoid anything associated with the indicated numbers. Happily enough, it does not concern our audience, consisting of a number of scientists from Europe and one from Japan.
Moreover, I believe that the knowledge and experience brought by Honourable Attendants as their valuable contribution will inevitably lead all of us excellent outcome, while this combination of 13 and 4 will have further great impact on our common success.
Sacramento, has an intersection where the 13th Street crosses the 13th Avenue, here, the Polish Histamine Research Society XIII Meeting crosses with Histamine H4 receptors!

I wish all of you - the Honourable Lecturers and Dear Participants much enjoyment from your presentations and personal communications, I wish valuable, vivid discussions.
Finally, I also wish much pleasure from associated social events.

Enjoy your participation in our Conference and your stay in Lodz and may it remain in your memory as a pleasant experience, prompting your comeback in two years time.

W. Agnieszka Fogel
President of the Polish Histamine Research Society



CONFERENCE PROGRAMM

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Hotel Ambasador, Lodz, Poland

	Thursday, 21.10.2010
15.00	Arrival, accommodation and registration, Hotel Ambasador, Kosynierów Gdyńskich 8 St., 93-320 Lodz
18:30	Opening Ceremony W. Agnieszka Fogel, President of the Polish Histamine Research Society
19:00	*Conference Lecture:* **HISTAMINE H_4 RECEPTORS: THE IONIC LOCK-MODEL ON LIGAND BINDING AND ACTIVATION,** H. Stark, Johann Wolfgang Goethe University Frankfurt am Main, Institute of Pharmaceutical Chemistry, Frankfurt, Germany
20:00	Dinner
	Friday, 22.10.2010
9:00-12:45	Session I, chaired by W. Agnieszka Fogel and Jerzy Jochem

Invited lecture:
THE ROLE OF HISTAMINERGIC SYSTEM IN WAKEFULNESS OF ZEBRAFISH,
P. Panula,
Neuroscience Center and Institute of Biomedicine, University of Helsinki

MONOAMINE OXIDASE ACTIVITIES IN THE LEFT AND RIGHT VENTRICLES OF HEARTS FROM ISCHEMIC AND NON ISCHEMIC END-STAGE CARDIOMYOPATHIES,
M. E. Manni, Ch. Nediani, E. Borchi, C. Giordano, G. D'Amati, E. Cerbai, L. Raimondi,
Department of Pharmacology, University of Florence, Italy; Department of Biochemical Sciences University of Florence, Italy; Department of Pathological Anatomy of the University "La Sapienza" of Rome, Italy

CENTRALLY ACTING LEPTIN-INDUCED RESUSCITATING EFFECT IN HAEMORRHAGE-SHOCKED RATS – AN INVOLVEMENT OF THE SYMPATHETIC NERVOUS SYSTEM,
J. Jochem, Z. Kalarus, A. Krawiec, L. Spaccapelo, A. Ottani, D. Giuliani, S. Guarini,
Department of Basic Medical Sciences, Medical University of Silesia, Bytom, Poland; Department of Cardiology, Congenital Heart Diseases and Electrotherapy, Medical University of Silesia, Silesian Center for Heart Diseases, Zabrze, Medical University of Silesia, Katowice, Poland; Department of Biomedical Sciences, Section of Pharmacology, University of Modena and Reggio Emilia, Modena, Italy

THE INFLUENCE OF ADJUVANT ARTHRITIS ON ISCHEMIA-REPERFUSION INJURY AND ISCHEMIC PRECONDITIONING IN RAT HEART,
M. Wojciechowska, M. Wątroba, G. Ciurzyńska, S. Maśliński,
Department of General & Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

3-IODOTYRONAMINE (T1AM) METABOLISM AND INSULIN RESISTANCE AT GLUCOSE AND FATTY ACID UPTAKE IN RAT WHITE ADIPOCYTES,
M. E. Manni, R. Zucchi, S. Zemniece, A. Saba, L. Raimondi,
Department of Pharmacology, University of Florence, Italy, Department of Human and Environmental Sciences University of Pisa, Italy; Department of Chemistry, University of Pisa, Italy

INVOLVEMENT OF HISTAMINERGIC MODULATION IN THE PROCESS OF STEM CELLS DIFFERENTIATION INTO INSULIN PRODUCING CELLS,
D. Szukiewicz,
Department of General & Experimental Pathology, First Department of Obstetrics & Gynecology, Second Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland

Coffee/tea

Invited lecture:
H_4 RECEPTORS AND LUNG DISEASE,
M. Ennis,
The Queen's University of Belfast, Nothern Ireland, UK

Poster presentation:
STUDY ON DRUGABILITY OF HISTAMINE H_4 RECEPTOR LIGANDS IN THE GROUP OF TRIAZINE DERIVATIVES,
A. Dymek, M. Więcek, J. Handzlik, E. Pękala, K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland

13:00 Lunch

14:30 -17:00 Session II, chaired by Katarzyna Kieć-Kononowicz and Krzysztof Walczyński

INTESTINAL TISSUE KALLIKREIN - KININ SYSTEM IN INFLAMMATORY BOWEL DISEASE,
A. Stadnicki, U. Mazurek,
Department of Basis Biomedical Sciences, Medical University of Silesia, Katowice, Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

MAST CELLS AND INFLAMMATORY BOWEL DISEASE,
J. Wejman, M. Pyzlak, D. Jarosz, W. Tarnowski, D. Szukiewicz,
Department of Pathology, Professor Witold Orlowski Clinical Hospital, Center for Medical Postgraduate Education, Warsaw, Poland, Department of General & Experimental Pathology, Medical University of Warsaw, Warsaw, Poland, Department of General & Gastrointestinal Surgery, Professor Witold Orlowski Clinical Hospital, Center for Medical Postgraduate Education, Warsaw, Poland

THE ROLE OF HISTAMINE IN INTEGRIN ALPHA-BETA$_3$ EXPRESSION WITHIN HUMAN TROPHOBLAST FROM HYPERTENSION-COMPLICATED PREGNANCIES,
G. Szewczyk, M. Pyzlak, W. Śmiertka, D. Szukiewicz,
Department of General & Experimental Pathology, Medical University of Warsaw, Warsaw, Poland, Department of Gynecological Oncology, Maria-Sklodowska Institute of Oncology, Warsaw, Poland

Poster presentations:

DIETHER DERIVATIVES AS HISTAMINE H$_3$ RECEPTOR ANTAGONISTS WITH ACETYL- AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY,
D. Łażewska, M. Ignasik, N. Guzior, B. Malawska, K. Kieć-Kononowicz,
Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Poland; Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Cracow, Poland

DETERMINATION OF ADME PROCESSES OF THE DL76 COMPOUND, A NEW NON-IMIDAZOLE HISTAMINE H$_3$ RECEPTOR ANTAGONIST,
M. Szafarz, J. Rząsa, J. Szymura-Oleksiak, D. Łażewska, K. Kieć-Kononowicz,
Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, Poland; Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland

NEW N-(4-PHENOXYALKYLPIPERAZIN-1-YL)ALKYL- AND PHENYLALKYLAMINE DERIVATIVES AS NON-IMIDAZOLE HISTAMINE H$_3$-ANTAGONISTS,
M. Staszewski and K. Walczyński,
Department of Synthesis and Technology of Drugs, Medical University of Lodz, Lodz, Poland

OREXIN TYPE-2 RECEPTOR INHIBITS CYCLIC AMP SYNTHESIS IN NEURONAL CELL CULTURES FROM RAT CEREBRAL CORTEX,
A. Urbańska, A. Woldan-Tambor, P. Sokołowska, M. Namiecińska, K. Biegańska, J. B. Zawilska,
Institute for Medical Biology Polish Academy of Sciences, Lodz, Poland, Department of Pharmacodynamics Medical University of Lodz, Lodz, Poland

HISTAMINE CONTENT IN DIABETIC PLACENTA AND EXPRESSION OF BRADYKININ RECEPTORS – POSSIBLE INDICATORS OF PROINFLAMMATORY CHANGES,
D. Szukiewicz, M. Pyzlak, A. Stangret, D. Bialoszewski, S. Maslinski,
Department of General & Experimental Pathology, First Department of Obstetrics & Gynecology, Second Faculty of Medicine, Medical University of Warsaw, Warsaw, 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, 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

17:00 Sandwich/Coffee/Tea

18:15 Bus transfer to Arthur Rubinstein Lodz Philharmony

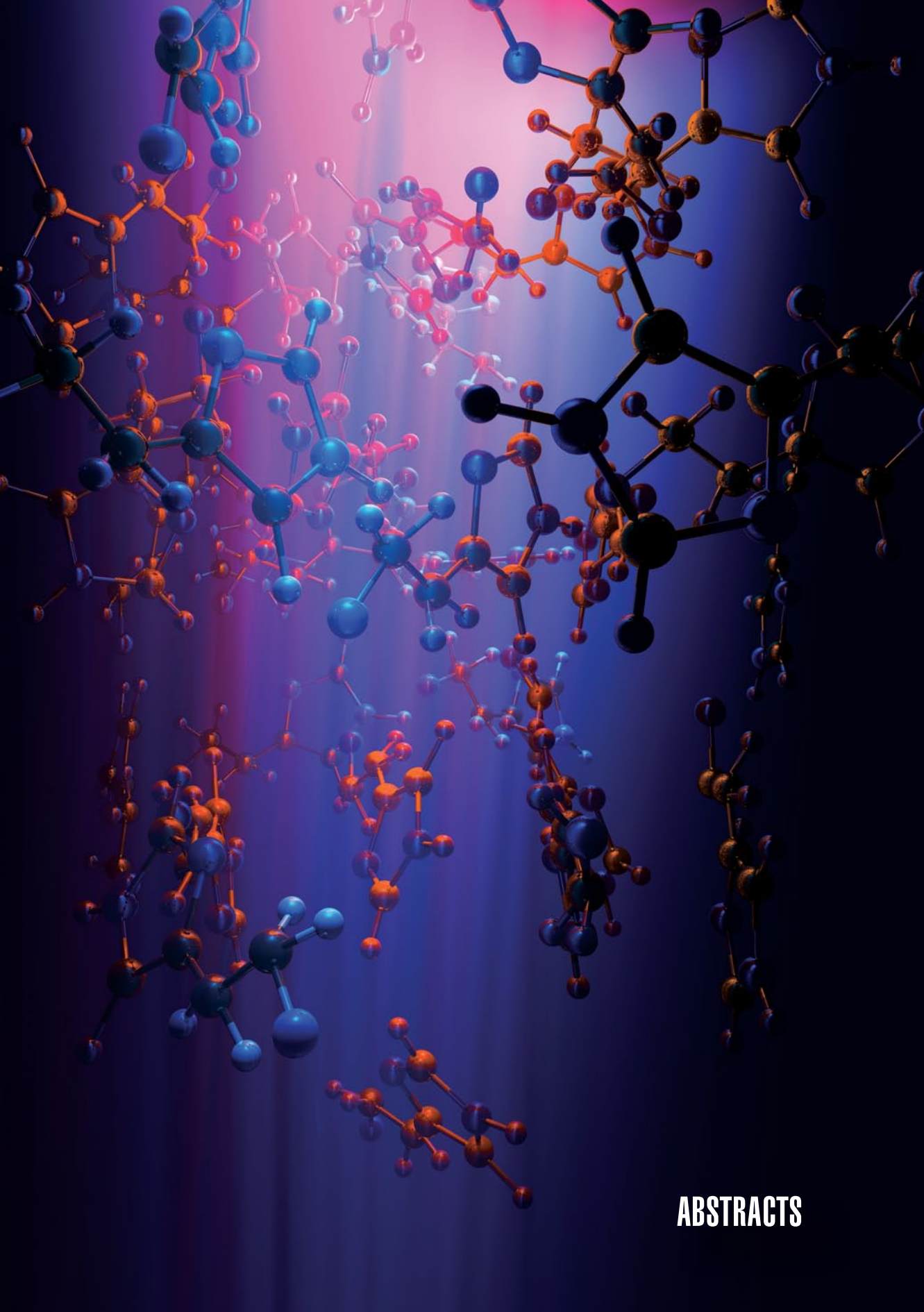
19:00 Symphony Concert: The Riddle of D-Minor,
F. Schubert - VIII Symphony H-moll “Unfinished”,
A. Bruckner – IX Symphony D-moll
Arthur Rubinstein Philharmonic Orchestra and Chorus, conductor: Marc Tardue

21:30 Dinner, Orfeusz Restaurant

Transfer back

CONFERENCE PROGRAMM

	Saturday, 23.10.2010
9:30-12:30	Session III, chaired by Madeleine Ennis and Piotr Thor
	Invited lecture: **ESTABLISHMENT OF A MODEL CULTURE SYSTEM FOR CUTANEOUS MAST CELLS,** S. Tanaka, Department of Immunochemistry, Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan
	Poster presentations:
	MAST CELLS AND LONG-TERM VAGUS NERVE STIMULATION IN THE DIET INDUCED OBESITY IN RATS - EXPERIMENTAL STUDIES, M. Kurnik, K. Gil, A. Bugajski, P. Thor, Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland
	SALSOLINOL ACTS ON MAST CELLS, INTERSTITIAL CELLS OF CAJAL AND MYENTERIC PLEXUS NEURONS IN THE RAT GUT, M. Kurnik, K. Gil, A. Bugajski, P. Thor, Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland
	TRANSCRIPTIONAL ACTIVITY OF MITOCHONDRIAL SUPEROXIDE DISMUTASE 2 GENE IN ANTERIOR LENS CAPSULE IN PATIENTS WITH PSEUDOEXFOLIATION SYNDROME, B. Strzałka-Mrozik, L. Prudło, U. Mazurek, W. Romaniuk, M. Kwiecień, Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland, Department of Ophthalmology, Medical University of Silesia, Sosnowiec, Poland
	INFLAMMATION IN BORRELIA BURGDORFERI INFECTION, S. Dudek, J. Gola, M. Kwiecień, U. Mazurek, Department of Molecular Biology, Medical University of Silesia, Katowice, Poland
	PORTOCAVALLY SHUNTED RATS PERFORM HOLE BOARD TEST BETTER THAN SHAM OPERATED RATS, A. Stasiak, M. Maksymowicz and W. A. Fogel, Department of Hormone Biochemistry Medical University of Lodz, Poland; Department of Surgical Research and Transplantology Polish Academy of Sciences Medical Research Center, Warsaw, Poland
	Closing Ceremony of the XIII-th Conference of the Polish Histamine Research Society
	Coffee/Tea
12:30	Lunch
14:30-17:00	COST BM0806 Members one-to-one discussion
18:30	Opera by Georges Bizet “Carmen”, Grand Theater in Lodz
21:30	Dinner



ABSTRACTS

HISTAMINE H_4 RECEPTORS: THE IONIC LOCK-MODEL ON LIGAND BINDING AND ACTIVATION

Holger Stark

Johann Wolfgang Goethe University Frankfurt am Main, Institute of Pharmaceutical Chemistry, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany; E-mail: h.stark@zafes.de

Like other histamine receptor subtypes the histamine H_4 receptor (H4R) belongs to the rhodopsin-like family of class A G-protein coupled receptors [1]. It also shares some sequence homology and pharmacological properties to its structurally related histamine H_3 receptor, e.g. high constitutive activity.

Recently we have developed a promising aminopyrimidine class of H4R ligands which differ a lot concerning their efficacy rates at H4Rs [2]. Depending on the substitution pattern the compounds vary from inverse agonism to partial agonist properties. Taking into account the different structural variations of these ligands in relationship to their binding mode we have performed a molecular dynamic simulation. This computational investigation revealed two different binding modes for partial and inverse agonists [3]. A "pseudo ionic lock" motif was detected as hydrogen bond network between transmembrane domain III and transmembrane domaine VI which may be relevant for receptor activation.

The development and optimization of the aminopyrimidine class will be discussed as well as the hypothesis on the different molecular binding modes of partial and inverse agonists at H4R.

References

1. *E. Tiligada et al. Histamine H3 and H4 Receptors as Novel Drug Targets. Expert Opin. Investig. Drugs 2009, 18, 1519-1531.*
2. *K. Sander et al. 2,4-Diaminopyrimidines as Histamine H4 Receptor Ligands – Scaffold Optimization and Pharmacological Characterization. Bioorg. Med. Chem. 2009, 17, 7186-7196.*
3. *T. Werner et al. In Silico Characterization of Different Ligand Binding Modes in the Human Histamine H4 Receptor and Their Impact on Receptor Activation. ChemBioChem 2010, 11, 1850-1855.*

THE ROLE OF HISTAMINERGIC SYSTEM IN WAKEFULNESS OF ZEBRAFISH

Pertti Panula, Hisaaki Kudo, Pauliina Tuominen, Stanislav Rozov, Maria Sundvik

Neuroscience Center and Institute of Biomedicine, University of Helsinki

Zebrafish has emerged as a useful experimental animal for developmental studies, but recent studies also show that it can be successfully used to analyze general mechanism of complex behaviors like sensorimotor regulation and sleep. Combination of good possibilities to combine genetic manipulation with detailed analysis of neuronal circuits in the brain and quantitative behavioral analysis has resulted in increasing knowledge of the roles of different neurotransmitters in these mechanisms. During the dark period, larval and adult zebrafish rest in a characteristic position and exhibit increased threshold to stimuli, characteristics of sleep. Three histamine receptors, corresponding to H1R, H2R and H3R, have been cloned in zebrafish. They are all expressed in the brain.

Zebrafish larvae aged 6-7 days showed distinct locomotor patterns: high spontaneous activity during the light period and low activity during the dark period. Inactivation of HDC by a morpholino oligonucleotide decreased brain histamine levels to below 10% of normal, and reduced locomotor activity during the light period. During the light period, normal larvae showed distinct rapid burst swimming activity when lights were shut off. This dark-induced flash response was completely abolished in hdc morphant fish. Both H1R and H3R were strongly expressed in the dorsal telencephalon, which was also the main target of histamine fibers in the brain. The target neurons expressed glutamatergic markers but not GABAergic markers. Anterograde tracing with DiI showed that major pathways from this target region are to contralateral dorsal telencephalon, medial basal hypothalamus and posterior hypothalamus, including direct innervation of the histaminergic neuorons. Thus, histamine in zebrafish seems to control sensorimotor activation through a hypothalamo-telencephalic pathway, which targets glutamatergic neurons. The two brain regions are reciprocally connected.

Supported by the Academy of Finland and the Sigrid Juselius Foundation.

MONOAMINE OXIDASE ACTIVITIES IN THE LEFT AND RIGHT VENTRICLES OF HEARTS FROM ISCHEMIC AND NON ISCHEMIC END-STAGE CARDIOMYOPATHIES

Maria Elena Manni[1], Chiara Nediani[2], Elisabetta Borchi[2], Carla Giordano[3], Giulia D'Amati[3], Elisabetta Cerbai[1], Laura Raimondi[1]

[1]Department of Pharmacology, University of Florence, Italy; [2]Department of Biochemical Sciences University of Florence, Italy; [3]Department of Pathological Anatomy of the University "La Sapienza" of Rome, Italy

Background. Heart failure (HF) is a cardiovascular syndrome representing an important and increasingly clinical problem. Notwithstanding the different ethiopatogenesis, at least three detrimental determinants are commonly found: unbalanced redox state, increased sympathethic tone and insulin-resistance. In the context of this trilogy, we thought important to evaluate the activity of mitochondrial monoamine oxidase (MAO; EC 1.4.3.4) in human failing hearts. These enzymes, deaminating catecholamine and serotonin may: i) locally increase reactive oxygen (hydrogen peroxide) and carbonyl species (aldehydes) ii) control substrate receptor-independent effects[1,2] and iii) modulate metabolic effects of substrates[3]. Accordingly, we aimed to measure the activities of MAO, catalase, aldehyde dehydrogenase-2 (ALDH-2) in biopsies of right (RV) and left ventricles (LF) from non failing (NF; n=4) and failing human hearts secondary to ischemic (*IHD: n=7*) and non ischemic (*not-IHD: n=5*) cardiomyopathies. In addition, in the same specimens we also evaluated the levels of expression of the serotonin transporter (SERT), malonyldialdehyde (MDA) and of carbonylated proteins as index of oxidative damage.

Methods. MAOs activity was assayed radiochemically using [^{14}C]-serotonin and [^{14}C]-benzylamine (100 μM, 1 μCi/ml) for MAO-A and B respectively. Instead, ALDH-2 and catalase activities, SERT protein expression were assayed spectrophotometrically and by Western-blot analysis respectively.

Results. In human NF and failing hearts, MAO-B predominated over MAO-A. Total MAO activity (A+B; nmoles/mg of protein/30 min) was 2.3 ± 0.1 in NF, 14.7 ± 1.0* in *IHD (*$p<0.001$ vs. NF) and 2.8 ± 0.1 in *not-IHD.* Moreover, while in *IHD*, no differences were evident between MAO-B and MAO-A activity in the LV over the RV (6.4 ±1.5 vs. 3.5 ± 0.9; $p>0.05$), in *not-IHD*, MAO-A was at higher levels in LV over the RV (0.77 ± 0.14 vs. 0.35 ± 0.11; *$p<0.05$). Catalase activity increased in failing with respect to *NF* hearts and it was higher in the LV over the RV of *IHD* (24.5 ± 4.5* vs. 15.7 ± 3; *$p<0.03$) and *not-IHD* (27.53 ± 4.34* vs. 17.18 ± 3.5; *$p<0.03$) hearts. ALDH-2 activity also increased in human failing hearts but no differences in its activity were observed between ventricles. MDA and carbonylated proteins levels were higher in failing than in *NF* hearts, with MDA levels significantly higher in RV vs. the LV in *not IHD* (3.5 ± 0.44* vs. 2.7 ± 0.44; $p<0.05$). SERT protein was detectable in each specimen. In respect of cardiomyopathy SERT expression increased in IHD hearts.

Our results indicate that in human HF, MAO activity might represent a fingerprint of cardiomyopathy. In fact, in *IHD*, total MAO activity was much higher than in NF hearts but the relative percentage of each isoform was similar to that of NF hearts. Instead, in *not-IHD* cardiomyopathy, while total MAO activity did not differ from NF, the percentage of MAO-A in the LV increases from 27% in NF to 47% in *not-IHD*. This finding associate with a parallel increase in catalase activity, likely accounting, in not-IHD cardiomyopathy, for the reduced MDA levels found in the LV in respect of the RV.

References

1. Villenueve et al., Am J Physiol Heart Circ Physiol. 297:H821; 2009.
2. Bianchi et al., Circulation. 112:3297; 2005.
3. Fischer et al., Biochem J. 311: 575; 1995.

CENTRALLY ACTING LEPTIN-INDUCED RESUSCITATING EFFECT IN HAEMORRHAGE-SHOCKED RATS – AN INVOLVEMENT OF THE SYMPATHETIC NERVOUS SYSTEM

Jerzy Jochem[1], Zbigniew Kalarus[2], Adam Krawiec[1], Luca Spaccapelo[3], Alessandra Ottani[3], Daniela Giuliani[3], Salvatore Guarini[3]

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[2]Department of Cardiology, Congenital Heart Diseases and Electrotherapy, Medical University of Silesia, Silesian Center for Heart Diseases, Szpitalna 2, Zabrze, Medical University of Silesia, Katowice, Poland;
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Leptin is a peptide hormone produced by adipocytes and, by centrally acting, it decreases adipose tissue mass through a reduction in appetite and increase in energy expenditure. The former effect is associated with centrally-mediated activation of the sympathetic nervous system, which also leads to a pressor action in normotensive rats. In our previous studies we demonstrated that several central neurotransmitters/neuromodulators which have anorexigenic properties, including α-MSH, ACTH and histamine, are able to activate compensatory mechanisms leading to resuscitating effect in haemorrhagic shock. The purpose of the present study was to examine haemodynamic effects of leptin intracerebroventricularly administered in haemorrhage-shocked rats. Moreover, we used peripheral adrenoceptor blockade to investigate an involvement of the sympathetic nervous system in leptin-induced effects. Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats subjected to severe haemorrhagic hypotension, with mean arterial pressure (MAP) stabilized at 20-25 mmHg, which resulted in the death of all control animals within 30 min. Leptin (20 μg/5 μl/rat) evoked long-lasting rises in MAP and heart rate (HR), with a subsequent increase in renal, mesenteric and hindquarters blood flows and a 100% survival at 2 h. MAP and regional haemodynamic effects were inhibited by a pre-treatment with the α_1- and α_2-adrenoceptor antagonists prazosin (0.5 mg/kg i.v.) and yohimbine (1 mg/kg i.v.), respectively. In contrast, the β-adrenoceptor antagonist propranolol (1 mg/kg i.v.) completely blocked leptin-induced HR changes, without influence on MAP and peripheral blood flows. In conclusion, the results of our haemodynamic studies demonstrate for the first time that centrally acting leptin induces a long-lasting pressor effect with an improvement in the survival rate at 2 h in rats subjected to haemorrhagic shock. The effect is associated with the activation of the sympathetic nervous system. We hypothesize that the resuscitating action can be associated with leptin-induced secretion of α-MSH and histamine which, as we previously demonstrated, also evoke anti-shock effects in the same model of severe hypovolaemia.

THE INFLUENCE OF ADJUVANT ARTHRITIS ON ISCHEMIA-REPERFUSION INJURY AND ISCHEMIC PRECONDITIONING IN RAT HEART

Małgorzata Wojciechowska, Mateusz Wątroba, Grażyna Ciurzyńska, Sławomir Maśliński

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Aim. Ischemic heart preconditioning (IPC) is the exposure of myocardial tissue to brief, repeated periods of ischemia (I) and reperfusion (R) in order to render the myocardium resistant to the deleterious effects of prolonged episodes of I and R.

Some of the inflammatory chemicals were proved to mimic IPC. On the other hand inflammation is negatively associated with the extent of ischemia-reperfusion injury (IRI). The aim of this study was to assess if adjuvant arthritis, which is a model of chronic inflammation has an influence on IPC and if adjuvant arthritis changes the extent of IRI in the rat heart.

Materials & Methods. All the experiments were made in situ rat hearts. Adjuvant arthritis was induced by complete Freund's Adjuvant subcutaneous injection. Experiments were made in 4 groups: in experimental rats with or without IPC before prolonged I and R (AA-IPC, AA respectively) and in control rats with or without IPC before prolonged I and R (C-IPC, C respectively). IPC was induced by two episodes of left coronary artery occlusions (3 and 7 minutes), separated by 5 minutes of reperfusion. Prolonged ischemia and reperfusion lasted 30 and 60 minutes respectively. In order to estimate the extent of IRI the infarct size and the severity of ventricular arrhythmias during prolonged ischemia were measured. Infarct size was expressed as the area of necrosis as a percentage of area at risk (tetrazolium staining).

Results. IPC reduced the area of necrosis from 58.9 ± 1.4% in group C to 25.6 ± 1.5% in group C-IPC ($p<0.05$) and reduced the incidence of ischemia induced ventricular tachycardia (VT) that developed in 71.4% of animals from group C and zero in group C-IPC ($p<0.05$). In experimental rats IPC did not change the area of necrosis: AA – 65.0 ± 2.2% and AA-IPC – 58.3 ± 2.6% (p about 2.5), but the incidence of VT decreased from 100% in group AA to zero in group AA-IPC ($p<0.05$). In animals from groups C and AA both area of necrosis and severity of ventricular arrhythmia were similar.

Conclusions. 1. Adjuvant arthritis has no influence on the extent of IRI caused by prolonged ischemia and reperfusion. 2. IPC has no positive effects on the extent of the infarct size in rats with adjuvant arthritis whereas its anti-arrhythmic effect is significant.

3-IODOTYRONAMINE (T1AM) METABOLISM AND INSULIN RESISTANCE AT GLUCOSE AND FATTY ACID UPTAKE IN RAT WHITE ADIPOCYTES

Maria Elena Manni[1], Riccardo Zucchi[2], Santa Zemniece[1], Alessandro Saba[3], Laura Raimondi[1]

[1]Department of Pharmacology, University of Florence, Italy;
[2]Department of Human and Environmental Sciences University of Pisa, Italy; [3]Department of Chemistry, University of Pisa, Italy

Background. Thyronamines are chemical relatives of thyroid hormones, among which, 3-iodothyronamine (T1AM) and thyronamine (T0AM), occur endogenously from decarboxylation and deiodination of thyroid hormones. Less is known on the significance of T1AM and T0AM tissue levels and whether they can be modulated at pathological conditions. *In vivo*, a single ip injection in mice of T_1AM rapidly decreases heart rate and core body temperature [1] whereas, in rats, a single-dose of T_1AM dramatically switches fuel utilization away from carbohydrates toward lipids [2]. Up to now it is not known whether T1AM metabolic effects at glucose and fatty acid metabolism may be reproduced in isolated cells and whether they involve T0AM production following deiodination of T1AM.

Aim. In rat white adipocytes, cells expressing high amineoxidases levels and which are insulin-sensitive we aimed to study: i) the kinetic of T1AM degradation by amine oxidases and the eventual T0AM production, ii) whether T1AM was able to modulate glucose and fatty acid uptake iii) whether inhibition of T1AM metabolism modified T1AM potency at energy substrate uptake.

Methods. T1AM and T0AM levels were assayed by HPLC coupled to tandem mass spectrometry [4] (MAO-B and Bz-SSAO activities were studied radiochemically, using appropriate [^{14}C]-benzylamine concentrations [5] and [^{14}C]-serotonin for MAO-A in the absence and in the presence of T1AM (from 0.1 to 10 μM). The identification of the amine oxidase involved was stated by running enzyme assays in the presence of selective inhibitors (semicarbazide 1 mM or pargyline 100 μM). Palmitate uptake was measured radiochemically using the non-metabolizable analog [^{3}H]-2-D-dideoxyglucose (1 μM; 1 mCi/ml) and [^{14}C]-palmitic acid (100 μM; mCi/ml) respectively [3].

Results. T1AM (10 μM) disappears from rat white adipocyte medium with $T_{1/2}$=2min, in the presence of semicarbazide, T1AM half-life increased significantly ($T_{1/2}$= 30 min). Moreover, T0AM is also time-dependently produced from T1AM. T1AM inhibits benzylamine oxidation by MAO-B and by Bz-SSAO with an IC50 (M) 6.5 x 10^{-6} and 3.3 x 10^{-6} respectively, whereas no inhibition is measured on serotonin oxidation.

T1AM (from 50 nM to 10 μM) does not affect the basal glucose or palmitate uptake. Instead, T1AM (from 50 nM to 10 μM inhibits insulin (20 nM) stimulated glucose and palmitate uptake with an IC50 (M) of 1.4 x 10^{-6} and 1.8 x 10^{-8} respectively. T1AM exerts non-competitive type of inhibition on insulin-stimulated glucose uptake. In the presence of pargyline and semicarbazide the IC50 (M) on insulin stimulated glucose and palmitate uptake shifts to 5.7 x 10^{-7} and 1.4 x 10^{-6} respectively.

Conclusions. Plasmalemma Bz-SSAO, mitochondrial MAO-B and a deiodinase are the enzymes involved in T1AM metabolism. Moreover, T1AM is endowed of direct metabolic effects reducing insulin stimulation at glucose and palmitate uptake. To this latter effect, and not at glucose uptake, seems to contribute T0AM too.

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INVOLVEMENT OF HISTAMINERGIC MODULATION IN THE PROCESS OF STEM CELLS DIFFERENTIATION INTO INSULIN PRODUCING CELLS

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Aim. Modulation of histamine receptors activity may be involved in the mechanism of stem cells proliferation and differentiation. Human amniotic epithelial cells (HAEC) maintain stem cell-like characteristics with ability to differentiate into all three germ layers: endoderm, mesoderm and ectoderm. Histidine decarboxylase as well as histamine H_1 and H_2 receptors are expressed in HAEC. The aim of the study was to examine the relationship between histamine concentration, H_1 and H_2 expression, and nicotinamide-induced differentiation of HAEC into pancreatic islet beta-like cells (PBLC).

Materials & Methods. Extraplacental membranes were obtained after normal term pregnancies (N=16). The amnion was manually separated from the chorion and HAEC were isolated using Okita's method in our modification. Briefly, amnion was washed with phosphate-buffered saline and incubated for 20 min in 5% trypsin diluted 1:250 (Gibco, UK) at 37^{0}C to remove adherent cell debris. After another, extended period of digestion (3% trypsin for 90 min at 37^{0}C) released HAEC were cultured in normoxia in 24-well culture plates (1.0 million cells per well) in Ham's F12 and Dulbeco's modified Eagle medium supplemented with 10% fetal calf serum at 37^{0}C in humidified atmosphere. Altogether, 96 cultures were established and divided into groups where effects of histamine (100μM) on the pancreatic differentiation of HAEC were investigated with/without histamine H_1 and H_2 receptor antagonists: mepyramine (10μM) and cimetidine (10μM), respectively. Nicotinamide was administered as a potent inducer of the pancreatic differentiation, except to control cultures. Every third day over a 15 day period the mean C-peptide concentration (MCPC) in the culture medium was determined immunoenzymatically (C-peptide ELISA kit; BioVendor, Germany) as a marker of differentiation into PBLC. At the same time, supernatants were removed and the cultures formalin-fixed and paraffin-embedded for H_1 and H_2 receptors immunostaining in 5μm sections. Quantitative immunohistochemistry was applied for evaluation of H_1 and H_2 expression.

Results. C-peptide was detected on Day 6 with the concentrations gradually increasing until Day 12. They then remained at virtually the same concentration (3.75-fold increase). Histamine significantly ($p < 0.05$) increased MCPC in a time dependent manner. Histamine H_2 receptor blockade significantly reduced histamine-related increase of C-peptide levels, whereas the H_1 receptor antagonist did not significantly affect MCPC in all cultures. Up to Day 9, the expression of the H_2 receptors were unchanged (compared to the control HAEC culture). After this time, they were significantly decreased ($p<0.05$) with the mean % value on Day 12 compared to control HAEC being 33.68. The differences in H_1 receptor expression were not significant over time.

Conclusion. Pancreatic differentiation of HAEC into beta-like cells may be augmented by histamine. Considering the role of histamine H_2 receptors in cell differentiation, variable expression of H_2 during nicotinamide-induced differentiation of HAEC into PBLC in vitro may define a time-point or threshold, indicating involvement of histamine at earlier stages of this process.

H4 RECEPTORS AND LUNG DISEASE

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H4 receptors were cloned in a number of different laboratories around 2000. Both message and protein are found in cells of the immune system such as mast cells, natural killer cells, monocytes, dendritic cells and eosinophils. Antagonists of the H4 receptor have profound effects on these cells such as inhibiting histamine-induced chemotaxis. Thus the H4 receptor could be a key player in the innate immune system.

In this talk I will review works showing the role of the H4 receptor in airways disease (asthma) and also postulate some other diseases where it might play a role. Atopic asthma is characterised by eosinophilia in the bronchoalveolar lavage fluid (BAL). In an acute murine asthma model, either the use of H4 receptor knockout mice or an H4 antagonist resulted in a reduction of total BAL cells and BAL eosinophils [1]. In contrast a recent study showed that the combination of an H1 and H4 receptor antagonist resulted in a synergistic inhibition of the eosinophilia, although both compounds alone were without significant effect. This suggests that the development of compounds with combined H1 and H4 efficacy could be useful [2]. A further characteristic of asthma is airway hyperreactivity; intratracheal administration of an H4 agonist not only reduced airway hyperreactivity but also reduced inflammation [3]. In the nose the presence of both H1 and H4 receptors is raised in nasal polyp tissue compared to that from nasal turbinate [4]. We have also managed to demonstrate the presence of H4 receptors on nasal epithelial cells. Airway epithelial cells are much studied in diseases such as cystic fibrosis and COPD, both situations where there are frequent infections. The function of H4 receptors on airway epithelial cells remains to be investigated.

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STUDY ON DRUGABILITY OF HISTAMINE H4 RECEPTOR LIGANDS IN THE GROUP OF TRIAZINE DERIVATIVES

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Aim. The recently characterized histamine receptor, named H_4 receptor (H_4R) is the promising target in the therapy of inflammatory diseases and disorders of the immune system [1]. To further explore the (patho) physiological function of the H_4R selective ligands are needed.

JNJ 7777120 (indole carboxamide derivative) is the first potent and selective H_4R antagonist used as reference compound to study the H_4R [2]. Many pharmaceutical companies and academic research groups have synthesized a large variety of high potent H_4R ligands [3, 4]. In the group of substituted 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives some compounds have been recently described in patent literature as potent modulators of H_4R [5].

In our previous studies, a number of H_4R ligands in the group of 1,3,5-triazine derivatives were obtained. The series of 4-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine derivatives with different substituents in 6-position was synthesized and tested for their affinities at hH_4R [6]. Nowadays, strong emphasis is put on eliminating non-druglike compounds at an early stage of drug development process [7]. In this context, toxicity- and phospholipidosis risk for these compounds were evaluated *in silico.* Selected compounds were also tested on their mutagenicity *in vitro* by using the *Vibrio harveyi* assay.

Materials & Methods. To assess the mutagenic activity *in vitro* four *Vibrio harveyi* strains were used: BB7 (natural isolate), BB7M (BB7 derivative containing *mucA* and *mucB* genes in the plasmid pAB91273, products of these genes enhance error-prone DNA repair), BB7X (BB7 derivative oversensitive to UV light) and BB7XM (created by transforming BB7X strain with the plasmid pAB91273). Phospholipidosis risk was predicted basing on pKa and clogP values predicted theoretically by the use of two computational programs, ADME/Tox (pKa) and OSIRIS (ClogP). Toxicity was evaluated by OSIRIS program.

Results. Investigated compounds showed different mutagenic properties *in vitro*. The compound with highest human H_4R affinity was devoided of the mutagenicity in this test.

Calculated pKa-ClogP values for all investigated triazine derivatives fit in those for drugs with negative phospholipidosis-inducing capacity, thus compounds displayed very low probability to cause the occurrence of a few foamy macrophages in the lung**.**

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INTESTINAL TISSUE
KALLIKREIN - KININ SYSTEM IN INFLAMMATORY BOWEL DISEASE

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Introduction & Aim. Kalikreins cleave kininogens to release kinins. Kinins exert their biological effect by activating constitutive bradykinin receptor -2 (BR2), which are rapidly desensitized, and inducible by inflammatory cytokines bradykinin receptor -1 (BR1), resistant to densensitization. Intestinal tissue kallikrein (ITK) may hydrolyze growth factors and peptides whereas kinins increase capillary permeability, evoke pain, stimulate synthesis of nitric oxide and cytokines. and promote adhesion molecule – neutrophil cascade. Thus activation of intestinal kallikrein – kinin system may have relevance to idiopathic inflammatory bowel disease (IBD).

Materials & Methods. The distribution and significance of the ITK – kinin components have been investigated by last ten years in experimental and human IBD.

Results. Our and others results have demonstrated that ITK is localized in intestinal goblet cells, and it is released into interstitial space during inflammation. Kallistatin, an inhibitor of tissue kallikrein, has been shown in epithelial and goblet cells, and was decreased in inflamed intestine as well as in plasma compared with noninflammatory controls. Alterations in both the distribution and levels of kinin receptors in intestinal tissue of IBD patients were demonstrated. B1R was upregulated in inflamed intestine, and has been found to be expressed in a basal part of normal intestine but in the apical portion of enterocytes in the inflamed tissue. In addition ITK and B1R (but not B2R) were visualized in macrophages forming granuloma in Crohn's disease. In animal studies B2R blockade decreased intestinal contraction, however had o limited effect on inflammatory lesions. Recent results documented B1R upregulation, in part dependent of TNF–α, in experimental enterocolitis, and demonstrated that selective, nonpeptide B1R receptor antagonist decreased morphological and biochemical features of intestinal inflammation. In addition both B1R and B2R have been indicated to mediate epithelial ion transport that leads to secretory diarrhea.

Conclusions. Taken together it seems that upregulation of B1R in human and animal intestinal inflammation provides a structural basis for the kinins function, and selective B1R antagonist may have potential in therapeutic trial of IBD patients.

MAST CELLS AND INFLAMMATORY BOWEL DISEASE

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Aim. The aim of our study was to describe morphometric characteristics of mast cells present in different parts of bowel wall in patients with inflammatory bowel disease (IBD).

Materials & Methods. We have analyzed full-thickness tissue fragments of intestinal wall taken from patients after colectomy choosing three main groups: 1. Crohn's disease (CD) 2. ulcerative colitis (CU) 3. control. First two groups consisted of patients who received surgical treatment in the course of inflammatory bowel disease. Control group consisted of patients who underwent colectomy as a treatment of non-IBD and non-vascular disorders. Tissue fragments from these patients were obtained from macroscopically unchanged bowel wall and were confirmed to have no pathologic changes microscopically. Each group consisted of 50 patients. All tissue fragments were paraffin embedded and stained histochemically and immunohistochemically according to appropriate protocols. We have used antibodies against tryptase to highlight mast cells and CD31 to evaluate vascular network. Slides from each case were independently examined by two experienced pathologists and evaluated with the use of morphometrics workstation based on ImageJ software.

Results. Our results show significantly larger number of mast cells infiltrating bowel wall in the course of IBD, when compared to control. Also differences in distribution of mast cells were shown. In patients with CD the number of mast cells located within mucosa was significantly higher when compared to control. The number of mast cells found in *muscularis propria* in patients with CD is also higher when compared with control and CU.

Conclusion. The pathology of IBD is not well understood. The role of mast cells in pathophysiology of IBD has not been sufficiently described in human yet. Studies based upon animal models indicate their role in maintaining of mucosal barrier. The role of histamine, tryptase and matrix metalloproteinases is currently investigated in the context of IBD. Our study shows that mast cells may indeed play an important role in pathophysiology of IBD. We've also managed to describe the localization of mast cells in tissue fragments containing full thickness of bowel wall, not only in superficial biopsy specimens.

THE ROLE OF HISTAMINE IN INTEGRIN ALPHA-BETA3 EXPRESSION WITHIN HUMAN TROPHOBLAST FROM HYPERTENSION-COMPLICATED PREGNANCIES

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Aim. Differentiation of trophoblast of developing placenta is directed into two separate pathways: villous and extravillous line. Extravillous trophoblast invades spiral arteries and replaces their endothelium. To succeed in this, the switch in surface proteins is necessary. Extravillous trophoblast is characterized with positive expression for alphav-beta3 integrin. The aim of the study was to examine if histamine can influence expression of alphav-beta3 integrin in cell culture from preeclamptic placenta. This was supported with the fact that histamine can influence expression of alphav-beta3 integrin in healthy placenta and histamine concentration in placenta is increased in preeclampsia.

Material & Methods. Placenta after Cesarean sections were used for the study and divided into two groups: physiological pregnancy (n=11) and pregnancy complicated with preeclampsia (n=11). Tissue samples were taken and cell cultures were conducted according to Kliman's metod. Alphav-beta3 integrin expression were examined after 48, 72 and 96 hours with ELISA.

Results. There was a significant ($p < 0.05$) increase of alphav-beta3 integrin expression in healthy placenta which was histamine-dependent, while in preeclamptic placentas the level of protein expression was not different from control group.

Conclusion. Histamine can stimulate alphav-beta3 integrin expression in healthy placenta, but fails in preeclampsia. This can be associated with depletion of either receptor or post-receptor mechanism.

DIETHER DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS WITH ACETYL- AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY

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Aim. Alzheimer's disease (AD) is a neurodegenerative disorder with multifactorial causes, characterized by a deficit in cholinergic function [1]. Inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), such as donepezil, galantamine or rivastigmine, improve cholinergic signaling in the central nervous system (CNS) and are widely prescribed as symptomatic treatments for AD [2]. Histamine H_3 receptors (H_3Rs) are mainly expressed in CNS, regulating histamine biosynthesis and release from histaminergic neurons. The blockade of H_3Rs increases brain histamine levels and the release of other neurotransmitters such as e.g. acetylcholine, dopamine or serotonin, involved in cognitive processes. Some of anti-H_3 ligands are present in clinical studies for treatment of AD (e.g. in phase II: ABT-288, GSK-239512, MK-0249 or PF-03654746) [3] but dual-acting compounds (AChE/BuChE inhibitors-anti H_3 ligands) might be a novel therapeutic approach for the symptomatic treatment of AD and might reduce side effects of recent therapy.

Materials & Methods. AChE and BuChE inhibitory activity was evaluated by spectrophotometrical Ellman's method using AChE from electric eel and BuChE from horse serum (2.5 units/1mL) [4].

Resuslts. The tested compounds acted as BuChE inhibitors with micromolar range (IC_{50} = 1.28 -15.87 μM). At the same time they displayed weak activity against AChE (IC_{50} = 7.91-74.62 μM). The obtained results were compared with anti-H_3R activity of compounds [5]. The most promising structure was 1-[3-(3-(4-chlorophenoxy)propoxy)propyl]azepane, which displayed IC_{50} values of 7.91 μM for AChE, 4.97 μM for BuChE, respectively and K_i of 3.48 nM for *h*H_3R.

Conclusion. Homopiperidine derivatives showed high binding affinities at recombinant human H_3R and moderate anti-cholinesterase activity.

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DETERMINATION OF ADME PROCESSES OF THE DL76 COMPOUND, A NEW NON-IMIDAZOLE HISTAMINE H3 RECEPTOR ANTAGONIST

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Aim. The biogenic amine histamine is implicated in the wide range of physiological and pathophysiological functions. The histamine H_3 receptor is acting as presynaptic auto- and heteroreceptor mainly in the CNS controlling the synthesis and release of histamine. Its activation modulates also the release of various important neurotransmitters such as dopamine, acetylocholine, norepinephrine, serotonin, GABA, glutamate and substance P which are involved in many brain functions including vigilance, memory processes, feeding behavior and locomotor activity. Antagonists of this receptor might be potentially useful in treatment of some neuropsychiatric diseases such as attention deficit hyperactivity disorder (ADHD), Alzheimer disease and schizophrenia [1, 2].

The aim of this pilot study was to evaluate absorption, distribution and elimination of DL76 (1-[3-(4-tert-butylophenoksy)propyl]piperidine) histamine H_3 receptor antagonist, which in preliminary pharmacological studies shows hKi equal 22 ± 3 nM and ED_{50} 2.8 ± 0.4 mg/kg, after intravenous (i.v.) and intragastric (i.g.) administration to rats in the single dose of 3 mg/kg.

Materials & Methods. Concentrations of DL76 compound in serum and tissues homogenates were determined using a high performance liquid chromatography system coupled with tandem mass spectrometer API 2000 (LC/MS/MS). Pharmacokinetic parameters were calculated by WinNonlin program (Pharsight, CA, USA).

Results. Basic pharmacokinetic parameters calculated after i.v. administrations showed the clearance of 217 mL/min/kg, volume of distribution 20 L and mean residence time (MRT) of 92 min. Absorption after i.g. administration can be described by bioavailability of 75% and mean absorption time (MAT) equal 0.7 h. Distribution of DL76 after i.v. administration was evaluated in brain, liver, kidneys and lungs. The highest area under the concentration-time curve, used as a measure of drug exposure, was observed for lungs (13519.25 ng*h/g), just slightly lower for brain (9418.92 ng*h/g) and the lowest for liver (1267.25 ng*h/g). Values of a disappearance constant were 0.25 h^{-1}, 0.41 h^{-1} and 0.16 h^{-1} for lungs, brain and liver respectively.

Conclusions. Compound DL 76 undergoes fast elimination. High volume of distribution may imply its intracellular character. Maximal exposure to the investigated compound can be observed in lungs and minimal in liver.

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NEW N-(4-PHENOXYALKYLPIPERAZIN-1-YL)ALKYL- AND PHENYLALKYLAMINE DERIVATIVES AS NON-IMIDAZOLE HISTAMINE H_3-ANTAGONISTS

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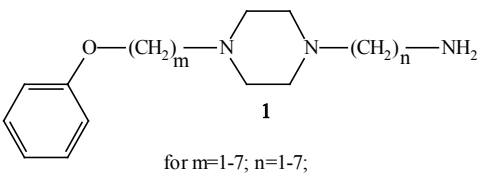
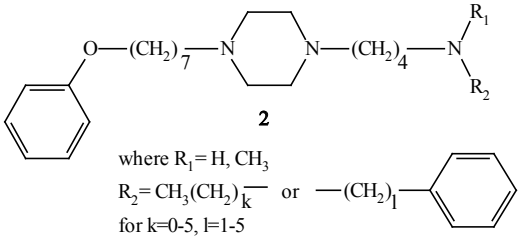
Background. The initial development of potent H_3 receptor antagonists in the early 1980s focused on extensive modification of the natural ligand histamine, and resulted in a series of very potent imidazole-containing H_3 antagonists. Based on these results it has been shown, that substitution of the imidazole nucleus is performed by a linking alkyl group, which posses another functionality e.g.: thiourea, isothiourea, guanidine, amidine, amine et cetera.. In most cases this polar functionality is additionally linked directly or by second alkyl spacer to a lipophilic moiety, which increases affinity, selectivity, and specifity. However, it has appeared that imidazole-containing ligands are associated with inhibition of cytochrome P450 enzymes, caused by imidazole nitrogen complexation to heme iron in the active site of the enzyme. For these reasons, the development of potent non-imidazole H_3 receptor antagonists was eagerly awaited
In the past fifteen years, a number of non-imidazole antagonists have since been reported. The successful replacement of the imidazole moiety with pyrrolidine, piperidine, piperazine and other basic tertiary amines was reported in patent applications and chemistry papers.

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 a new series of N-(4-phenoxyalkylpiperazin-1-yl)amines **1** and N-(4-phenoxyalkylpiperazin-1-yl)alkyl- and phenylalkylamines **2** as H_3 histamine receptor antagonists.



1

for m=1-7; n=1-7;



2

where R_1= H, CH_3

R_2= $CH_3(CH_2)_k$ or $-(CH_2)_l-$

for k=0-5, l=1-5

Results & Conclusion. The presented series of N-(4-phenoxyalkylpiperazin-1-yl)alkyl- and phenylalkylamine derivatives (**2**) all possess, moderate H_3-receptor antagonist potency.

In the first step, in order to optimize the structure, we have prepared a series of 1-[3-(4-phenoxyalkylpiperazinyl-1-yl)propyl)]amines (**1**). We studied the influence of the chain length of the alkyl spacer: a) between nitrogen at position 4 of piperazine and phenoxy moiety from 1 to7 methylene groups, retaining the constant distance (3 methylene groups) and b) between nitrogen at position 1 of piperazine and primary amine functionality. The most potent compound of this series is N-[5-[4-(7-phenoxyheptyl)piperazine-1-yl]butyl)]amine (pA_2=7.18). In the next step, the series of N-(4-phenoxyalkylpiperazin-1-yl)alkyl- and phenylalkylamine derivatives were synthesized. The most potent compound of these series is N-[5-[4-(7-phenoxyheptyl)piperazine-1-yl]butyl)]-N,N-methylethylamine (pA_2=7.28).

OREXIN TYPE-2 RECEPTOR INHIBITS CYCLIC AMP SYNTHESIS IN NEURONAL CELL CULTURES FROM RAT CEREBRAL CORTEX

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Background. Orexins (orexin A and B), also known as hypocretins (hypocretin 1 and 2), are multifunctional neuropeptides discovered by two research groups in 1998. Both orexins are derived from a common precursor (preproorexin) by proteolytic cleavage. Orexin-containing neurons project from the lateral hypothalamus to numerous brain regions, and are involved in the regulation of vigilance and sleep/wake cycle, feeding, appetite, and metabolic processes. Orexins act on their targets via two specific, membrane-bound, G protein-coupled receptors: OX_1 and OX_2. In contrast to numerous data on anatomy of orexin-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 stimulation of orexin receptors on cyclic AMP formation in primary neuronal cell 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. Orexin A (an agonist of OX_1 and OX_2 receptors) and [Ala^{11}-*D*-Leu^{15}]orexin B (a selective agonist of OX_2 receptors) did not significantly affect basal cyclic AMP formation in cortical neuronal cell cultures. Both peptides inhibited, in a concentration-dependent manner, the increase in the nucleotide production evoked by forskolin (a direct activator of adenylyl cyclase; 1 mM), pituitary adenylate cyclase-activating polypeptide (PACAP27; 0.1 mM) and vasoactive intestinal peptide (VIP; 3 mM). The inhibitory effect of orexin A on forskolin-, PACAP27-, and VIP-stimulated cyclic AMP synthesis was blocked by TCS OX2 29 (a selective antagonist of OX_2 receptors) and not affected by SB 408124 (a selective antagonist of OX_1 receptors). Pretreatment of neuronal cell cultures with pertussis toxin (100 ng/ml) abolished the studied effect of orexin A on cyclic AMP production.

Conclusion. It is suggested that in the primary neuronal cell cultures orexins, acting at OX_2 receptors coupled to PTX-sensitive G_i protein, inhibit cyclic AMP synthesis.

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HISTAMINE CONTENT IN DIABETIC PLACENTA AND EXPRESSION OF BRADYKININ RECEPTORS – POSSIBLE INDICATORS OF PROINFLAMMATORY CHANGES

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Aim. Increased concentration of histamine and elevated levels of bradykinin were reported in placental tissue obtained after diabetic pregnancies. Histamine contributes to cytokine participation in inflammation and allergy, whereas bradykinin - another well known proinflammatory mediator - induces mast cell degranulation, leading to increase in the local histamine concentration. Bradykinin B1 receptor shows strong involvement in the inflammatory response, whereas the B2 receptor mediates most of the effects induced by kinins. The aim of this study was to examine comparatively correlation between placental histamine concentration and placental expression of B1 and B2 in diabetes class C versus uncomplicated pregnancy.

Materials & Methods. Fourteen diabetic placentae were compared with 14 normal placentae (Group I and II, respectively). Histamine concentrations in placental cuts were estimated fluorimetrically. Expression of B1 and B2 was examined in immunostained paraffin sections by quantitative morphometry in the areas matched in mean vascular density.

Results. Histamine concentration in Group I was significantly ($p < 0.05$) increased compared to Group II (388 ±24.7 versus 237 ±15.1 ng/g of wet weight ±SEM). Mean expression of the B1 was augmented in diabetes and reached 291.4% of the value for Group II ($p<0.05$). The differences in mean expression of B2 receptors were non significant.

Conclusion. Increased amounts of histamine in placental tissue in diabetes producing pro-inflammatory conditions may change vascular properties to the some degree by influence on bradykinin receptors expression and vice versa (i.e. bradykinin may affect action of histamine). Pro-inflammatory reactions mediated via B1 should be expected rather, than changed vasomotor reactivity related to B2. Angiogenic properties of histamine and kinins should also be considered.

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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Aim. 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.

Materials & 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.

Conclusions. All differences were found to be statistically significant. Swine dysentery induces changes only in lymphocyte B subpopulation, but tissue levels of both studied neuropeptides changes in response to intestinal inflammation.

ESTABLISHMENT OF A MODEL CULTURE SYSTEM FOR CUTANEOUS MAST CELLS

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Accumulating evidence indicates that mast cells not only play critical roles in immediate allergic responses but also modulate a wide variety of immune responses including contact hypersensitivity and autoimmune diseases. Since mast cells originate from hematopoietic stem cells and their terminal differentiation occurs in the tissues, in which they are ultimately resident, it should be required for better understanding of physiological roles of tissue mast cells to pay a particular attention to their microenvironments. We established a model culture system that could mimic the murine cutaneous mast cells, in which IL-3-dependent bone marrow-derived primary cultured mast cells (BMMCs) were co-cultured with Swiss 3T3 fibroblasts in the presence of stem cell factor (SCF). The characteristics of mature cutaneous mast cells, such as the presence of Safranin staining-positive granules, and degranulation in response to cationic secretagogues and substance P, were acquired during the co-culture period. We determined the gene expression profile during the co-culture period and identified the candidate genes that are involved in regulation of mast cell maturation. Among these genes, we characterized *CD44*, which encodes one of the dominant receptor for hyaluronan, since *CD44* was one of the genes that were up-regulated during the co-culture period. A series of studies using the *CD44*$^{-/-}$ mice suggest that CD44 regulates cutaneous mast cell number through regulation of proliferation of immature mast cells. Our model culture system should contribute to better understanding of tissue mast cells and their specific functions involved in modulation of local immune responses.

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MAST CELLS AND LONG-TERM VAGUS NERVE STIMULATION IN DIET INDUCED OBESITY IN RATS – EXPERIMENTAL STUDIES

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Aim. Vagus nerve plays an important role in the regulation of food intake and body weight acting through several mechanisms - regulation of gut motility, secretion and absorption. There is an increasing evidence that mast cells play a general transduction role, as a neural relay. Mast cells - nerve associations have already been found in the myocardium, diaphragm, brain, gallbladder and skin in a variety of animals. The aim of this study was to investigate peripheral effects of chronic vagus nerve stimulation (VNS) on the mast cells number in the stomach, duodenum and proximal colon in rats.

Materials & Methods. Male Wistar rats (n=18) with mean body weight of 370 ± 20 g were kept during the whole study (100 days) on high calorie diet. Three groups of rats were used: with active microchip (MC) connected by electrodes with the left vagus nerve (MC group, n=6); animals with inactive MC without the electrodes (sham, n=6) and intact group - rats without MC and electrodes. Left vagus nerve was stimulated by rectangular pulses duration 10ms, amplitude 200mV, frequency 0.05 Hz generated by MC. After finishing the experiments tissue samples were taken from stomach, duodenum and proximal colon. Specimens were toluidine blue stained and mast cells were counted in mucosa, muscularis externa and serosa. The total number and percentage of degranulated mast cells were evaluated.

Results.

1. VNS significantly increased total mast cells number in the stomach compared to sham and intact groups - 282.2 ± 168 in the MC group, 192.3 ± 103.7 in the sham group and 188.7 ± 57.2 in the intact group.

2. The highest values of gastric mast cells were observed in muscularis externa - 180 ± 98.9 in the MC group, 131.5 ± 67.8 in the sham group and 124.3 ± 41.14 in the intact group. No statistically significant differences were found in mucosa and serosa.

3. The percentage of degranulated mast cells remained unchanged in all three layers of the stomach wall, however the highest values were observed in muscularis externa - 70.14 ± 7.43 in the MC group, 72.28 ± 9.15 in the sham group and 61.79 ± 9.77 in the intact group.

4. In the duodenum mast cells number after VNS (16.0 ± 9.05) remained unchanged compared to sham (18.0 ± 8.6) and intact group (23.7 ± 22.8).

5. In the proximal colon mast cells number after VNS (18.7 ± 13.4) remained unchanged compared to sham (17.3 ± 11.6) and intact group (22.3 ± 22.1).

Conclusions. Increase in the mast cell number after long-term vagus nerve stimulation demonstrates an important role of mast cells in the transition of the satiety signals sent by afferent vagus fibers. Thus, by learning how to modulate the mast cell - vagus axis we may gain better control over pathological conditions of gastrointestinal tract.

SALSOLINOL ACTS ON MAST CELLS, INTERSTITIAL CELLS OF CAJAL AND MYENTERIC PLEXUS NEURONS IN THE RAT GUT

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Aim. Mast cells in the gastrointestinal tract have been found in close spatial contact with the regulatory cells of gastrointestinal motility: interstitial cells of Cajal (ICC) and myenteric neurons, suggesting their functional interaction. Interstitial Cells of Cajal located mainly between the circular and longitudinal layer of the muscular coat are recognized as the pacemaker cells of gastrointestinal tract. The main function of the ICC is to generate electrical activity and organize phasic contractions in the gastrointestinal tract, and to modulate activity of myenteric neurons. Because of the regulatory role of mast cells, ICC and myenteric neurons even the slight damage or change in activity of these cells may cause considerable disorder of the gut motility. The catechol isoquinoline derivatives are endogenous compounds present in the mammalian brain and the representative one is referred to as salsolinol - 1-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline. Increased salsolinol levels are detected in the cerebrospinal fluid of Parkinson's disease patients. Gastrointestinal dysmotility in those patients has been partly associated with peripheral action of salsolinol.

The aim of this study was to evaluate salsolinol effects on mast cells, intramuscular interstitial cells of Cajal and myenteric neurons in the gastrointestinal tract of rats.

Materials & Methods. Male Wistar rats (n=10) were injected intraperitoneally with salsolinol (50 mg/kg/day) for 3 weeks and the equal group served as a control. On the last day the animals were sacrificed, stomachs, small and large intestines were removed, and paraffin embedded specimens were prepared. Slides were toluidine blue stained and mast cells were counted. The total number and percentage of degranulated mast cells were evaluated. ICC and myenteric neurons were visualized using anti c-Kit (CD117) and anti – PGP 9.5 rabbit polyclonal antibodies (Santa Cruz Biotechnology, USA), respectively and the LSAB/HRP/DAB staining kit (DAKO, USA). Gastric antral, duodenal and ascending colon intramuscular c-Kit positive cells and myenteric neurons were assessed by image analysis.

Results.

1. The total number of mast cells in the gastrointestinal wall was decreased in the salsolinol group compared to the control - in the stomach 98.7 ± 53.3 vs. 156.7 ± 45.8, in the duodenum 2.6 ± 2.1 vs. 7.83 ± 7.8 and in colon 12.8 ± 14 vs.10.7 ± 17,1 (salsolinol treated vs. control group).

2. In the examined group the surface area of the cells PGP 9.5 positive was lower in all examined stripes of gastrointestinal tract in comparison with control group in the stomach by 51%, in duodenum by 55%, and in colon by 45%.

3. The area of cells stained with anti c-kit (i.e. cells of Cajal) was lower by 28% in the stomach, by 19% in the duodenum, but no differences were observed in examined rats in the expression of c-kit in the colon in comparison with control group.

Conclusions. Carried out examinations showed the destructive action of salsolinol on the mast cells, muscular plexus neurons and the interstitial cells of Cajal in all segments examined of gastrointestinal tract.

TRANSCRIPTIONAL ACTIVITY OF MITOCHONDRIAL SUPEROXIDE DISMUTASE 2 GENE IN ANTERIOR LENS CAPSULE IN PATIENTS WITH PSEUDOEXFOLIATION SYNDROME

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Aim. To determine the transcriptional activity of mitochondrial superoxide dismutase 2 gene (SOD2), in the anterior lens capsule in patients with pseudoexfoliation syndrome(PEX).

Materials & Methods. The examined group consisted of 20 patients: 9 women, 11 man in an average age of 74,5 years (63-80) with diagnosed PEX syndrome and cataract. The control group consisted of 23 patients: 8 women and 15 men with average age of 71 (66-80) who were diagnosed to have cataract demanding surgery interference in the form of its removal without leaving any recognition of pseudo-exfoliation. Total RNA was isolated from the specimens applying commercially available kits (Total RNA Prep Plus A&A Biotechnology;) according to manufacturer's instruction. Transcriptional activity of SOD2 gene was evaluated on the basis of real time QRT-PCR technique with a SYBR Green I chemistry with using an Opticon™ DNA Engine Sequence Detector. Specify of RT-PCR reaction was confirmed by separating of RT-PCR products on 6% polyacrylamide gels, their visualizing with silver salts, and determining characteristic temperature of melting of each amplimer.

Results. SOD2 mRNA was detected in all the anterior lens capsule in patients with PEX syndrome (Me= 23288.3 copies/µgRNA) and in the control group (Me= 2600.0 copies/µgRNA). SOD2 mRNA expression was found to be significantly higher (Mann-Whitney U test, p=0.0015) in the group of patients with PEX syndrome in comparison with the control group.

Conclusions. Increased expression of mitochondrial superoxide dismutase 2 in the anterior capsule lens of patients with pseudoexfoliation syndrome may prove the oxidative stress role in the etiopathogenesis of this disorder.

INFLAMMATION IN BORRELIA BURGDORFERI INFECTION

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Aim. Toll-like receptors (TLR) play a significant role in the induction of inflammatory processes in the infected organism. They have been recognized as playing a fundamental role in activating innate immunity by recognizing specific bacterial patterns - PRR (Pathogen Recognition Receptor). It was confirmed experimentally that the response to histamine receptors enhances TLR-2 and TLR-4 expression during the Gram (-) and Gram (+) infection.

Materials & Methods. Using oligonucleotide microarray HG-U133A (Affymetrix) we determined the expression of genes encoding histamine and proinflammatory factors in the case of spirochete infection Gram (-) Borrelia burgdorferi that causes Lyme disease. Material was peripheral blood of patients with Lyme-induced joint type of spirochete: Borrelia burgdorferi sensu stricto.

Results. An increased expression of genes encoding TLR-2, TLR-4 and TLR-5, histamine, and proinflammatory factors in relation to persons not infected by borrelia spirochetes was demonstrated in Lyme disease.

PORTOCAVALLY SHUNTED RATS PERFORM HOLE BOARD TEST BETTER THAN SHAM OPERATED RATS

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Aim. The hole board (HB) test, an open-field spatial learning test that measures short- and long- term memory, defined as working (WM) and reference memory (RM), respectively, was employed to identify the effect of portocaval shunt on rat cognitive function. Portocavally shunted rats are widely used as an animal model of hepatic encephalopathy.

Materials & Methods. Rats with portocaval anastomosis (n = 13) and sham operated counterparts (n = 10) were used in the HB test consisting of habituation, training and retest [1]. The habituation phase lasted 4 consecutive days. The rats were food deprived but watered *ad libitum*. At trial, lasting long enough to collect all food pellets, but no more than 10 min for any rat, all 4 x 4 equidistant holes were baited with food (50 mg each). The monitoring comprised the number of visits to the baited holes. *Finding of less than 15/16 pellets on the 4th day was the 1st exclusion criterion. T*raining started in 3 days after habituation was completed, its duration being 7 consecutive days with a pause on the 5th and on the 6th day. The rats were trained to collect pellets from 4 holes only. The time period for that trial was either 5 min or it was the time needed to collect 4 food pellets. One training session consisted of 4 consecutive trials/rat, with 1 min apart (cleaning and baiting the same chosen holes with pellets). Registration: the number of hole visits. *2nd exclusion criterion: WM ratio =/<50%, RM ratio =/< 40% at the 7th day.* Only the rats that positively passed the defined criteria were further examined. Retest was done on both groups without any intervention and following amnestic treatment. Scopolamine, a centrally acting muscarinic cholinergic antagonist, was administered in dose of 1 mg/kg i.p., in 30 min before food search task to produce memory deficit.

Results. All the shunted rats (13/13) passed the inclusion criteria, while 3/10 sham operated rats had to be withdrawn from further testing. The shunted rats performed better vs. the sham operated ones and tended to demonstrate better results in WM evaluation (64.63 ± 3.66% *vs*. 58.49 ± 2.68%), while RM ratio was similar. PCA rats had also shorter latency time (i.e. the time between the trial onset and the first hole visit) vs. the sham-operated controls (1.19 ± 0.07 s *vs*. 2.39 ± 0.68 s) and needed shorter time to collect all the pellets (83.73 ± 3.92 s *vs*. 108.21 ± 12.51 s). Scopolamine significantly increased the time period needed to complete the trial by the sham (over twice as much) with a lower effect on PCA (*ca* 1.6) rats.

Conclusion. The obtained results indicate enhanced cognition and learning in the shunted rats. This could be related, at least in part, to their increased brain histaminergic activity [2]. On the other hand, considering the significant increase in food intake demonstrated by PCA rats, further examination employing another test, not based on food search task would be appropriate before drawing of any final conclusion could be attempted.

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