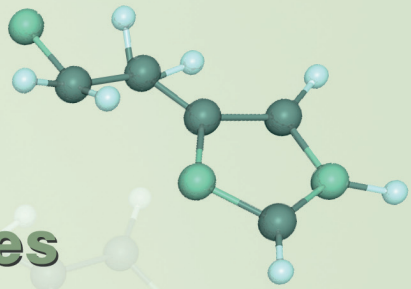
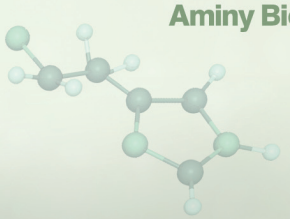
XI-th Conference of the Polish Histamine Research Society

XI 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

**Kazimierz Dolny, Poland**
**October 26-28, 2006**

*Dear Participant,*

*I would like to welcome you warmly and thank you for your coming and your valuable contributions.*

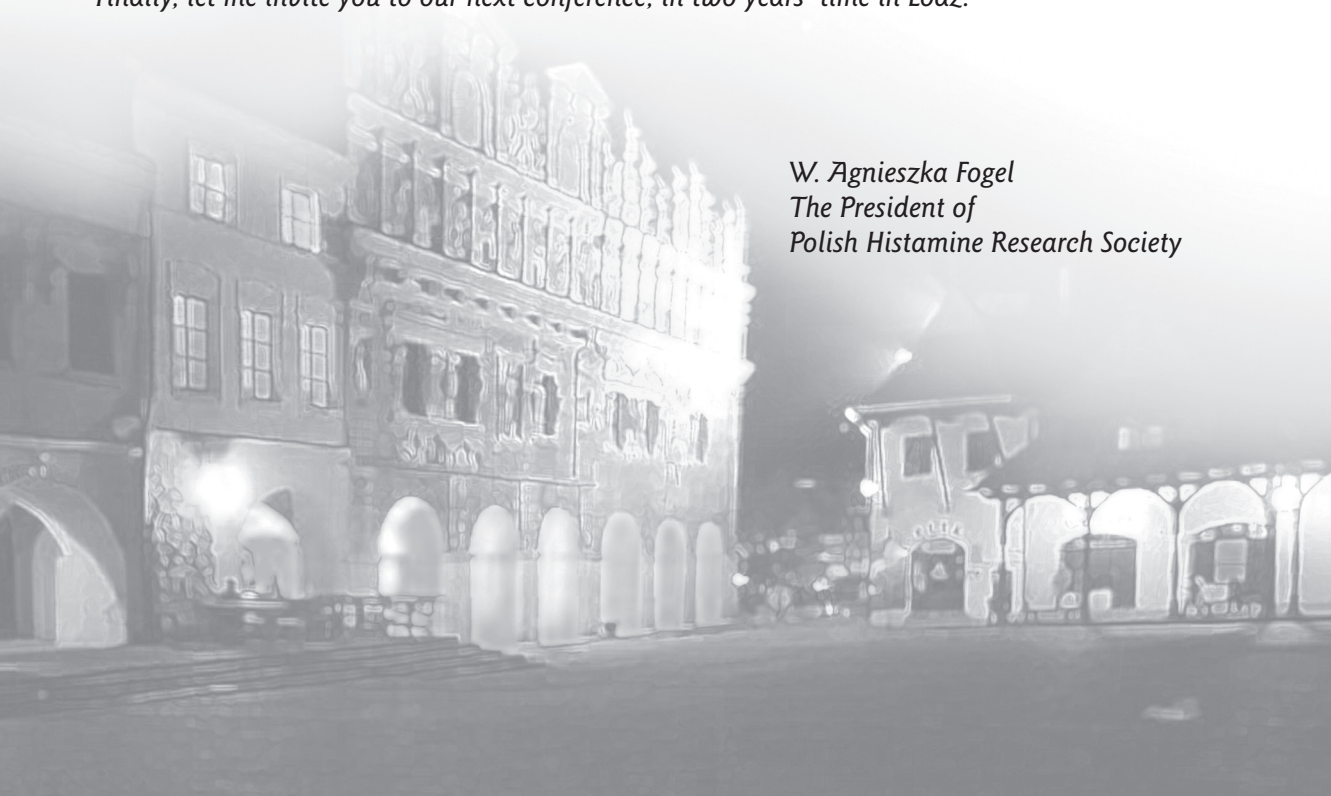
*We are here again after 16 years in this lovely town and even in the same conference hotel. We are here, exactly as in September 1990, to present some recent findings and discuss the state of art of knowledge on biogenic amines and other biologically active small molecular weight endogenous substances.*

*We speak different languages: Polish, German, Italian, Japanese but we can communicate using English. Likewise, we speak different professional languages having different scientific backgrounds, i.e. chemistry, biochemistry, molecular biology, pharmacology, medicine but we have common study subjects and can share our personal knowledge and experience with each other. No doubts, the multidisciplinary approach is the key to successful passage through labyrinth of unrevealed yet physiological and pathophysiological events regulation.*

*I wish the presenters and listeners much satisfaction from delivered papers and I am sure, the discussions will be lively and interesting.*

*In your free time, please, enjoy the unique, charming atmosphere of Kazimierz town, founded by our great King Casimir III in the fourteenth century.*

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

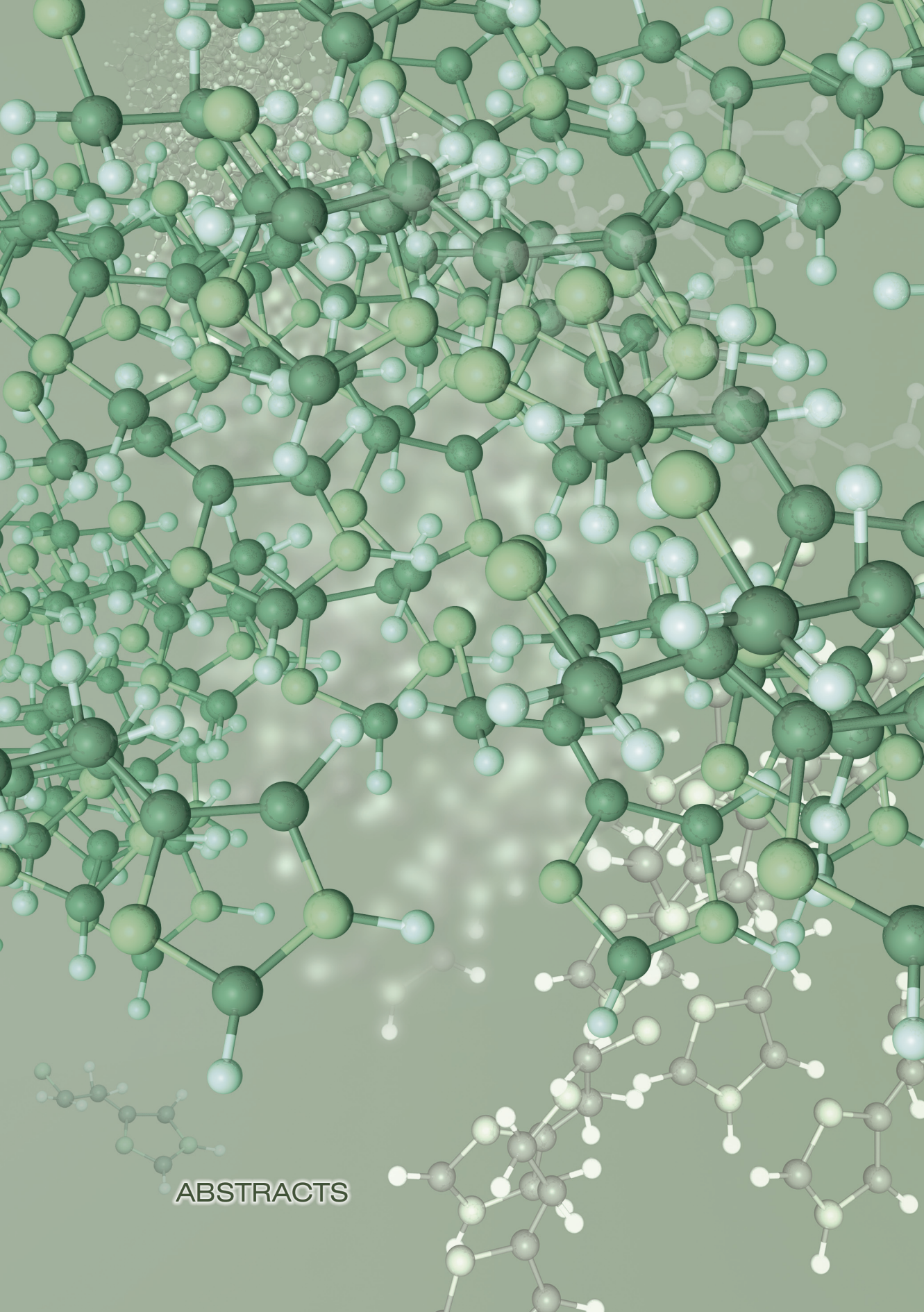


*W. Agnieszka Fogel*
*The President of*
*Polish Histamine Research Society*

**Conference Programm**

| | |
|---|---|
| ***Thursday, 26.10.2006*** | |
| 18:00 - 19:30 | ArrivalAccommodation in Zajazd Piastowski, Kazimierz Dolny (www.zajazdpiastowski.pl) |
| | Opening addresses<br>*Professor W.Agnieszka Fogel, President of the Polish Histamine Society*<br>*Professor Andrzej Lewinski, Rector of Medical University of Lodz*<br><br>*Presentation of Honorary Membership of the Society to Professor Walter Schunack, introduced by Professors Katarzyna Kieć-Kononowicz & Agnieszka Fogel* |
| | Conference Lecture<br>*Walter Schunack:*<br>"Histamine $H_3$-Receptor Antagonists: Drugs and Therapeutic Indications" |
| 20:00 - 22:00 | Dinner |
| ***Friday 27. 10.2006*** | |
| 9:00 -11:00 | Session I: Chaired by: *M. Karbownik-Lewinska and D. Szukiewicz*<br><br>HISTAMINE-RELATED APOPTOSIS AND OVARIAN FOLLICLES DEVELOPMENT by D. Szukiewicz, D. Maslinska, G. Szewczyk, J. Klimkiewicz, Medical University of Warsaw & Polish Academy of Sciences, Warsaw, Poland<br><br>COMPARISON OF TRANSCRIPTION ACTIVITY OF THE GENES ENCODING PLACENTAL BIOGENIC AMINE TRANSPORTERS AND RECEPTORS IN PREGNANCY COMPLICATED BY HYPERTENSION AND DIABETES by *A.Więcławek, G. Janikowska, U. Mazurek, H. Sławska, J. Szota,* Medical University of Silesia, Katowice, Poland<br><br>EXPRESSION PROFILES OF GENES ENCODING BIOGENIC AMINE TRANSPORTERS AND RECEPTORS IN HUMAN PLACENTA; NORMAL VERSUS COMPLICATED PREGNANCY *by H. Sławska, A. Więcławek, G. Janikowska, U. Mazurek, A. Owczarek, J. Szota*, Medical University of Silesia, Katowice, Poland<br><br>MICROARRAY ANALYSIS OF CYPs EXPRESSION IN PLACENTA<br>*G. Janikowska, A. Więcławek, U. Mazurek, H. Sławska, J. Szota, A. Owczarek,*<br>Medical University of Silesia, Katowice, Poland<br><br>CARDIOVASCULAR REACTIVITY IN A RAT MODEL OF PREECLAMPSIA – PRELIMINARY DATA by *J. Jochem, E.Olearska, K. Żwirska-Korczala,*<br>Medical University of Silesia, Zabrze, Poland<br><br>LOCALIZATION OF HISTAMINE H4 RECEPTOR IN HUMAN SYNOVIAL TISSUE FROM PATIENTS WITH RHEUMATOID ARTHRITIS.<br>*A.Grzybowska-Kowalczyk, E. Wojtecka-Lukasik, D. Maslinska, S. Maslinski*<br>Institute of Rheumatology & Polish Academy of Sciences & Medical University of Warsaw, Warsaw, Poland<br><br>INTERLEUKIN-8 (IL-8) STIMULATES GROWTH OF OVARIAN FOLLICLES AND ENHANCES THE OVULATION RATE IN VITRO by *D. Szukiewicz, D. Maslinska, M. Pyzlak, M. Wojciechowska,*<br>Medical University of Warsaw & Polish Academy of Sciences, Warsaw, Poland<br><br>EFFECT OF HISTAMINE CHLORAMINE ON LUMINOL-DEPENDENT CHEMILUMINESCENCE OF GRANULOCYTES by E Wojtecka-Lukasik, A. Grzybowska-Kowalczyk, D Maslinska, S Maslinski, Institute of Rheumatology & Polish Academy of Sciences & Medical University of Warsaw, Warsaw, Poland |
| 10:40-11:00 | Coffee/tea |

| | |
|---|---|
| 11:00-12:30 | Session II chaired by: *B. Skrzydlo-Radomanska and J.Jochem*<br><br>CASPASE-MEDAITED ACTIVATION OF HISTIDINE DECARBOXYLASE IN A MOUSE MASTOCYTOMA, P-815 by *S.Tanaka,* Mukogawa Women's University, Japan<br><br>SIGNAL TRANSDUCTION MECHANISMS UNDERLYING THYMOSIN B4 MAST CELLS ACTIVATION<br>*J. Wyczółkowska, A. Walczak-Drzewiecka, J. Dastych, W. Wagner,*<br>Polish Academy of Sciences, Lodz, Poland<br><br>EFFECTS OF PACAP, VIP, AND SOME RELATED PEPTIDES, ON<br>CYCLIC AMP GENERATING SYSTEM IN RAT NEURONAL AND ASTROCYTE CELL CULTURES<br>*M. Jóźwiak-Bębenista, Bednarek K., Nowak J.Z.,*<br>Department of Pharmacology, Medical University of Lodz, Poland<br><br>EFFECTS OF ADRENALINE ON CYCLIC AMP GENERATING SYSTEM AND EXPRESSION OF VEGF AND IL-8 IN HUMAN ENDOTHELIAL CELLS<br>*M. Namiecińska, A.Wiktorowska-Owczarek, J.Z.Nowak,* Polish Academy of Sciences &, Medical University of Lodz, Lodz, Poland; |
| 12:30-13:00 | Break |
| 13:00 -17:00 | Introduction to history of Janowiec and Kazimierz by the Town Majors |
| 20:00-23:00 | Dinner |
| ***Saturday, 28.05.2005*** | Session III chaired by: *B. Malinowska and E. Sewerynek* |
| 9:00-11:00 | CARDIAC EFFECTS OF THE ACTIVATION OF THE LOW AFFINITY STATE OF $\beta_1$-ADRENOCEPTORS IN PITHED RATS by *B. Malinowska, A. Zakrzeska, H. Kozłowska, E. Schlicker,* Medical University of Białystok, Poland & University of Bonn, Germany.<br><br>BUPRANOLOL – AN OLD DRUG WITH NEW PERSPECTIVES by *D.Żelaszczyk, H. Kozłowska, U. Baranowska, K. Kieć-Kononowicz, B.Malinowska, E. Schlicker,* Medical College of Jagiellonian University Cracow & Medical University of Białystok, Poland & University of Bonn, Germany<br><br>NEW $N^G$-ACYLATED IMIDAZOLYLPROPYLGUANIDINES AS POTENTIAL SELECTIVE HISTAMINE $H_2$-RECEPTOR AGONISTS by *M. Opuch, A. Kraus, P. Igel, S. Elz, R. Seifert, A. Buschauer, K. Kieć-kononowicz,* COLLEGIUM MEDICUM JAGIELLONIAN UNIVERSITY, KRAKÓW, POLAND & UNIVERSITY OF REGENSBURG, GERMANY<br><br>LIPOPHILICITY PARAMETERS OF DIFFERENT PIPERIDINE DERIVATIVES AS HISTAMINE $H_3$ RECEPTOR ANTAGONISTS by *K. Kuder, K. Kieć- Kononowicz, W. Schunack, J-Ch Schwartz, X. Ligneau, H. Stark,* Collegium Medicum, Jagiellonian University, Kraków, Poland & Freie Universität Berlin, Germany & [3] Bioprojet Biotech, Saint Grégorie Cedex, France & Johann Wolfgang Universität, Biozentrum, Frankfurt am Main, Germany<br><br>BENEFICIAL EFFECTS OF A PLANT HISTAMINASE IN A RAT MODEL OF SPLANCHNIC ARTERY OCCLUSION AND REPERFUSION, by *E. Masini, S Cuzzocrea, D Bani, E Mazzon, C Mujf, R Mastroianni, F Fabrizi, P Pietrangeli, L Marcocci, B. Mondově, PFMannaioni and R Federico,* University of Florence & University of Messina & IRCCS & University of Rome "La Sapienza"& C.N.R.& 3rd University of Rome, Italy<br><br>EFFECTS OF ANTIHISTAMINE TREATMENT ON ULCERATIVE COLITIS, BASED ON RAT MODEL by W. A. Fogel, Medical University of Lodz, Poland<br><br>EFFECTS OF MELATONIN ON ADRIAMYCIN-INDUCED CARDIOTOXICITY IN RATS<br>*by K. Dąbrowska, M. Stuss, J. Gromadzińska, W. Wąsowicz, E. Sewerynek,* Medical University of Lodz & The Nofer Institute of Occupational Medicine, Lodz, Poland |
| | Concluding remarks and Closing<br><br>Tea/coffee<br>General Assembly |
| 12:30-13:30 | Lunch<br>Departure |



# ABSTRACTS

# HISTAMINE-RELATED APOPTOSIS AND OVARIAN FOLLICLES DEVELOPMENT.

**Dariusz Szukiewicz[1,2], Danuta Maslinska[1,3], Grzegorz Szewczyk[1], Jakub Klimkiewicz[1]**

[1] Chair and Department of General & Experimental Pathology,
[2] Chair and Department of Obstetrics & Gynecology, 2nd Faculty of Medicine, Medical University of Warsaw, Warsaw, POLAND
[3] Institute of Medical Research Centre, Polish Academy of Sciences, Warsaw, POLAND

AIM: Our previous studies and reports of independent authors have indicated an involvement of histamine (HA) acting through histaminergic $H_1$ and $H_2$ receptors in follicular development and ovulation in vitro. Changes in HA release within ovarian tissue during the estrus seems to be related to cyclic changes of mast cells (MC) content and distribution in the ovary. Considering pro-apoptotic effects of HA as well as the fact, that most of the follicles in the ovary undergo atresia by apoptosis of somatic and germ cells, here we examined influence of HA administration on ovarian follicles development and ovulation in vitro, with a special respect to apoptosis.

MATERIAL & METHODS: Ovarian preantral follicles (N=240) of 150-180μm isolated from 12 immature mice (strain BALB/CanNCrlCmd) were individually cultured in culture plate inserts using method described by Nayudu and Osborn. The medium contained 5% immature mouse serum, supplemented with 100 mIU/ml recombinant human FSH, 5 ng/ml epidermal growth factor, 10 ng/ml insulin, 5 ng/ml fibroblast growth factor, 3 mM L-glutamine, 2.5 μg/ml transferrin, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 4 ng/ml selenium (as sodium selenite). The incubation took place under a humidified atmosphere of 4% $CO_2$ and 96% air at 37°C. Diameter of the follicles was measured daily using x100 magnification. The effects of HA (10 mmol/l) and $H_1$, $H_2$ blockade (mepyramine 20 μmol/l and cimetidine 10 μmol/l, respectively) on apoptosis were examined daily in respective groups on Day 3 (subgroups I.A and II.A) and Day 5 (subgroups II.A and II.B). Appropriate controls have been prepared for each group. Apoptosis within the follicles was determined by the TUNEL method (terminal deoxynucleotidyl transferase-mediated dUTP nick end- labeling). Microscopic images were analysed in the sections of ovarian follicles using computed morphometry for quantitative analysis (Quantimet 500+C, Leica Ltd, UK). At the end of culture period (Day 5th or Day 6th), the follicles were stimulated to ovulate with hCG (5 IU/ml Chorulon, Intervet, The Netherlands). At the and of the 24h incubation period ovulation rate was examined. Results were expressed as median percentages with interquartile ranges. Mann-Whitney's U test was applied and differences were accepted significant at $p < 0.05$.

RESULTS: HA administration in the preovulatory period significantly increased apoptosis (up to 188.54 median % of intensity observed in controls in Day 3) as well as inhibited follicular growth, reducing median daily increase in follicular size. Despite its pro-apoptotic effect, HA administered in the culture matured for ovulation significantly increased hCG-induced ovulation rate (31% versus 19% in controls; $p < 0.05$). Histaminergic blockade canceled or significantly reduced influence of HA on apoptosis, follicular growth and ovulation.

CONCLUSION: We found previously, that growing follicles showed increasing expressions of $H_1$ and $H_2$ as well as that the follicles matured for ovulation demonstrated the highest $H_1$ and $H_2$ expressions. The results from our current research suggest that, depending on the developmental stage, HA acting as apoptosis inducer via $H_1$ and $H_2$ receptors may play a role in the selection process of the dominant follicle, and stimulate ovulation. Knowing that HA stimulates progesterone synthesis via $H_2$ receptors, the importance of an appropriate balance between $H_2$ receptors stimulation, progesterone concentration and progesterone receptors expression deserves further studies.

# COMPARISON OF TRANSCRIPTION ACTIVITY OF THE GENES ENCODING PLACENTAL BIOGENIC AMINE TRANSPORTERS AND RECEPTORS IN PREGNANCY COMPLICATED BY HYPERTENSION AND DIABETES.

**Agnieszka Więcławek[1], Grażyna Janikowska[2], Urszula Mazurek[1], Helena Sławska[3], Justyna Szota[1]**

Medical University of Silesia in Katowice,
[1] Division of Molecular Biology,
[2] Division of Analytical Chemistry,
[3] Department and Clinic of Perinatology and Gynecology

Aim: The aim of this study was to compare expression of genes encoding transporters and receptors of biogenic amines such as norepinephrine, epinephrine, dopamine and serotonin in placenta of patients with pregnancy induced hypertension (PIH) and diabetes (PD).
Materials and methods: Frozen placentas (n=12) of which 7 were collected from PIH patients and 5 from patients with diabetes were homogenised and total RNA was extracted using Trizol® (Invitrogen) Reagent. Affymetrix microarrays were used to estimate the transcription activity of genes.
Results: Affymetrix microarray data were normalized by using RMA Express program. Subsequently, normalized data were analysed by Statistica 6.0 and Cluster 3.0 programs. The obtained results are presented on the following figure.

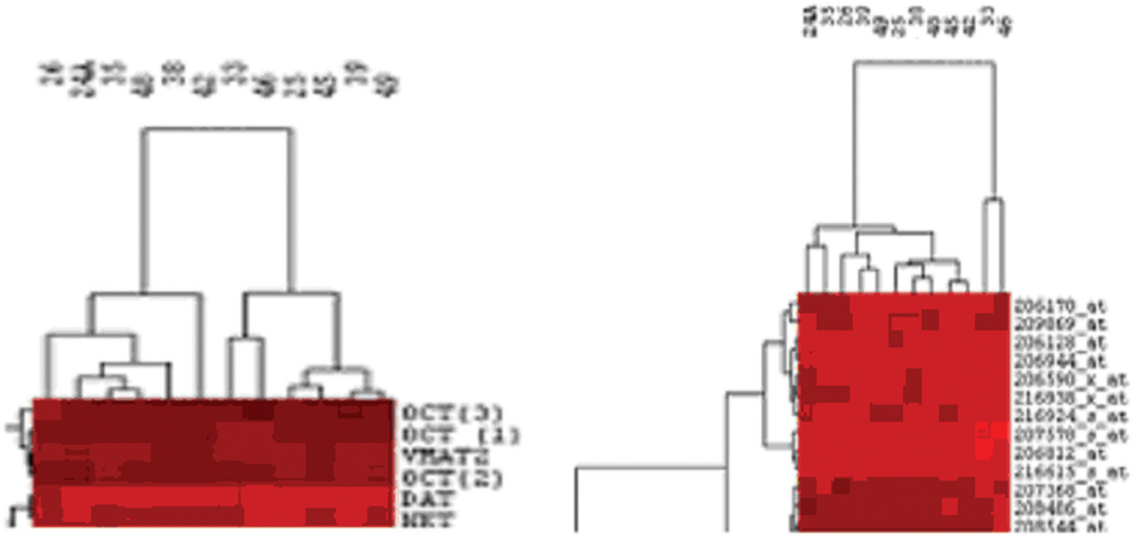



*Fig 1. Cluster analysis of genes encoding transporters (a) and receptors (b) of biogenic amines in placenta after pregnancies complicated by hypertension (samples number: 24A, 25, 33, 35, 42, 45, 49) and diabetes (26, 38, 39, 46, 48).*

The cluster analysis suggests that the expression profiles of genes encoding the transporters and receptors of biogenic amines in examined placentas of the two groups of patients are similar and grouped together. As further confirmed by regression analysis of data no genes which differentiate placenta with hypertension and diabetes could be found.
Conclusion: The reason of PIH is unknown. Some conditions, among them diabetes, can enhance a risk of its development. Accordingly, similar expression of genes encoding transporters (fig.1a) and receptors (fig.1b) for biogenic amines in PIH and PD placenta might suggest that molecular changes precede clinical symptoms of hypertension.

# EXPRESSION PROFILES OF GENES ENCODING BIOGENIC AMINE TRANSPORTERS AND RECEPTORS IN HUMAN PLACENTA; NORMAL VERSUS COMPLICATED PREGNANCY

**Helena Sławska[1] Agnieszka Więcławek[2], Grażyna Janikowska [3], Urszula Mazurek[2], Aleksander Owczarek[4], Justyna Szota[2]**

[1] Department and Clinic of Perinatology and Gynecology,
[2] Division of Molecular Biology,
[3] Division of Analytical Chemistry,
[4] Department and Division of Bionic,Medical University of Silesia, Katowice

Hypertension in pregnancy is either idiopathic or due to renal disease. A number of renal diseases may be causative, the most common being diabetic glomerulosclerosis. Pathogenesis of pregnancy-induced hypertension (PIH) is still not fully explained, however, among risk factors gestational diabetes keeps an important position and biogenic amine systems have strongly been implicated in pathomechanisms. In preeclampsia, for example, large quantities of circulating biogenic amines were reported to be associated with lowered expression of their transporters as well as lower catalytic efficiency of degrading enzyme, monoamine oxidase A.

The aim of this study was to compare expression profile of genes that encoded transporters and receptors of biogenic amines in placenta from normal pregnancies with these with hypertension and diabetes.

Collected frozen placental tissue was homogenised and the total RNA was extracted using Trizol ® Reagent. Transcription activity of genes was analysed with the Affymetrix microarrays. The data from 15 microarrays: 7– pregnancy-induced hypertension, 5 - diabetic pregnancies, 3-controls were normalized using RMA Express program. Subsequently, normalized data were analysed by Data Mining Tool and MicroArray Suite 5.0 and Cluster 3.0 programs.

Among transcripts differentiating between placentas after pregnancy-induced hypertension (PIH) and controls special attention deserve the transcripts encoding norepinephrine transporter (NET) and adrenergic receptors (AR).
Three out of four transcripts encoding NET present on microarray HG-U 133A are being candidates to differentiating transcripts.

Results obtained with Bland-Altman method for transcripts encoding adrenergic receptor ARA2C are showing that increased, in relation to the controls, expression of this receptor in placentas of patients with pregnancy-induced hypertension does not influence NET expression. Obtained results confirm at this point some previous investigations. From two transcripts encoding NET and being candidates to differentiating ones both show lower expression in diabetic placenta in relation to the controls. Similarly as in the placentas from PIH patients also in diabetic placentas a diminished expression of NET accompanies enlarged quantity of circulating amines, however, no change in the expression of adrenergic receptors in this group is present.

Summing up, the diminished expression of NET and the lowered catabolic activities (analysis of monoamine oxidase MAO-A) accompany predisposition to diabetic pregnancies or evident symptoms of pregnancy-induced hypertension.

# CARDIOVASCULAR REACTIVITY IN A RAT MODEL OF PREECLAMPSIA – PRELIMINARY DATA

**Jerzy Jochem, Ewa Olearska, Krystyna Żwirska-Korczala**

Department of Physiology, Medical University of Silesia, H. Jordana 19, 41-808 Zabrze, Poland,

E-mail: jjochem@poczta.onet.pl, tel./fax: +48 32 272 23 62

Preeclampsia is estimated to affect approximately 5-10% of all pregnancies and it is a leading cause of maternal mortality and a major contributor to maternal and perinatal morbidity. However, the mechanisms underlying the pathogenesis of this disease are still not clear. There are many experimental models of preeclampsia, including the reduced uterine artery perfusion pressure model, deoxycorticosterone acetate-induced preeclampsia and NG-nitro-L-arginine-methyl ester-induced preeclampsia. In the present study, we used a model of high-sodium intake model of preeclampsia to study the cardiovascular reactivity to histamine and noradrenaline. In this model, the pregnant rats are given 1.8% NaCl solution during the last of the 3 weeks of gestation [1]. The NaCl-supplemented pregnant rats exhibit several symptoms that are characteristic for preeclampsia, including an increase in mean arterial pressure (MAP), fetal growth retardation, proteinuria and a decrease in plasma renin activity [1]. Our studies were carried out in ketamine/xylazine anaesthetised female Wistar rats weighing 250-320 g. We measured MAP, heart rate (HR) and mesenteric blood flow using the pressure transducer RMN-201 (Temed, Poland) and Transit Time Flowmeter type 700 (Hugo Sachs Elektronik, Germany), respectively. The preliminary data show that intravenous (iv) bolus injection of histamine (2.5-20 μg/kg) produced significantly higher effects (decreases in MAP, and mesenteric vascular resistance [MVR], increases in HR) in non-pregnant and the control pregnant rats than in rats with NaCl-induced preeclampsia. In contrast, noradrenaline (0.5-15 μg/kg; iv) evoked significantly higher effects (increases in MAP and MVR) in rats with preeclampsia than in the other tested animals. In conclusion, increased sodium intake influences vascular responsiveness in pregnant rats.

[1] Beausejour A, Auger K, St-Louis J, Brochu M. High-sodium intake prevents pregnancy-induced decrease of blood pressure in the rat. Am J Physiol 2003; 285: H375-H383.

# LOCALIZATION OF HISTAMINE H4 RECEPTOR IN HUMAN SYNOVIAL TISSUE FROM PATIENTS WITH RHEUMATOID ARTHRITIS.

**Agnieszka Grzybowska-Kowalczyk[1], Elzbieta Wojtecka-Lukasik[1], Danuta Maslinska[2], Slawomir Maslinski[1,3]**

[1] Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland,
[2] Department of Experimental and Clinical Neuropathology, Medical Research Centre, Warsaw, Poland,
[3] Department of Pathophysiology, Medical University of Warsaw, Poland.

Aim: Rheumatoid arthritis (RA), a chronic inflammatory disease, is characterized mainly by immunological disturbances in synovial tissue, but its pathogenesis remains unclear.
Very little is known about the biological function of the novel histamine H4 receptor (H4R) and the recent experiments in vivo indicate its role in inflammatory conditions and immune system disturbances. There is also an evidence that H4 receptor plays a key role in chemotaxis of eosinophils and mast cells to amplify histamine-mediated allergic reactions. Expression of H4R has been observed in eosinophils, T cells, dendritic cells, basophils, and mast cells in blood and many organs.
While the presence of H4 receptor in cultured synovial cells of RA patients has been previously documented (Ikawa Y. Biol Pharm Bull 2005), distribution pattern of this receptor in synovial tissue slides has not been determined as yet.

Materials & Methods: Synovium samples were obtained at joint replacement surgery of RA patients or from young adults as control. Samples were fixed in 4% (v/v) paraformaldehyde and H4R was localized by immunohistochemical staining using the primary polyclonal antibodies.
RT-PCR technique confirmed the H4R specific mRNA expression in examined specimens. Results: We demonstrated the presence of H4 protein in various types of synovium cells. Histamine receptor H4 was found in the synoviocytes of the superficial layer membrane of synovium and synovial villi. H4R was also localized in cells of vascular walls and inflammatory cells infiltrated perivascular area.

Conclusion: Localization of histamine H4 receptor in synoviocytes of inflamed areas and also in vascular wall cells suggests that this receptor protein is essential in the course of inflammatory process during RA. Precise localization of H4 receptor in RA patients opens potential new avenues to investigate histamine-mediated mechanisms and to develop pharmacotheraputic agents to attenuate the disease.

# INTERLEUKIN-8 (IL-8) STIMULATES GROWTH OF OVARIAN FOLLICLES AND ENHANCES THE OVULATION RATE IN VITRO.

**Dariusz Szukiewicz[1,2], Danuta Maslinska[1,3], Michał Pyzlak[1], Malgorzata Wojciechowska[1]**

[1] Chair and Department of General & Experimental Pathology,

[2] Chair and Department of Obstetrics & Gynecology, 2nd Faculty of Medicine, Medical University of Warsaw, Warsaw, POLAND

[3] Institute of Medical Research Centre, Polish Academy of Sciences, Warsaw, POLAND

AIM: Based on in vivo studies, IL-8 can be considered an important regulatory factor in the ovary. Mast cells (MC) produce IL-8. The number of IL-8 positive cells is subjected to changes during the estrus and corresponds with MC. This fact may indicate an involvement of MC-derived IL-8 in local regulation of ovarian function. We examined influence of IL-8 on the development and ovulation of ovarian follicles cultured in vitro.

MATERIALS & METHODS: Based on a method used by Nayudu and Osborn, we developed an in vitro model with cultured murine follicles. Using dissecting microscope, preantral follicles (N = 160) of 150-180 μm were mechanically isolated from ovaries of immature 7-8 day old mice and individually cultured in culture plate inserts under a humidified atmosphere (4% $CO_2$ and 96% air) at 37 °C. The culture medium contained 5% immature mouse serum, 100 mIU/ml recombinant human FSH, 10 ng/ml insulin, 5 ng/ml epidermal growth factor, 5 ng/ml fibroblast growth factor, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 3 mM L-glutamine, 2.5 μg/ml transferrin, and 4 ng/ml selenium (as sodium selenite). Diameter of follicles was measured daily using x100 magnification and a calibrated micrometer. Starting from Day 3, the medium was supplemented with IL-8 (100 ng/ml) or IL-8 and the noncompetitive allosteric blocker of IL-8 receptors CXCR1/R2 (repertaxin; 100 nM; Sigma-Aldrich, USA) in group I and II, respectively. The follicles in group III were cultured without IL-8 and/or repertaxin. After 5-6 days, follicles from group II and group III were induced to ovulate with hCG (5 IU/ml Chorulon, Intervet, The Netherlands). In group I, the ovulation rate (OR) was estimated in subgroups: IA - without stimulation by hCG, IB - with hCG. Twenty-four hours period was chosen for ovulation assessment. Confidence intervals for the means were calculated to examine variation between samples. Mann- Whitney's U test was applied for statistical analysis of the daily diameter change of follicles and ovulation rates. Differences were considered significant at $p < 0.05$.

RESULTS: Supplementation of the culture medium with IL-8 produced an acceleration of follicular growth. This effect was abolished by administration of IL-8 receptors blocker, CXCR1/R2. Mean daily increase (% ±SD) in follicular size was significantly higher in group I, compared both to control group III, and repertaxin-treated group II (30.04 ±5.57 versus 18.79 ±4.36 and 19.21 ±4.19, respectively). The highest OR was observed in group IB and was significantly greater than in groups II and III (28% vs. 14% and 17%, respectively). Lack of hCG stimulation in group IA produced a significant decrease in OR value, despite presence of IL-8.

CONCLUSION: The results indicated that IL-8 can stimulate follicular growth and, together with hCG, can increase OR in vitro. This is one of the first studies demonstrating the role of IL-8 in ovarian follicle growth and ovulation in vitro. Previously, the role of IL-8 in ovulation and luteinization has been studied in vivo. Ovulation can be compared to a localized inflammatory reaction, because of the similarity of the compounds involved. Considering that reproductive hormones, especially estradiol, cause degranulation of MC, the role of other MC-derived mediators (e.g. cytokines) should be examined in the future.

# EFFECT OF HISTAMINE CHLORAMINE ON LUMINOL-DEPENDENT CHEMILUMINESCENCE OF GRANULOCYTES.

**Elzbieta Wojtecka-Lukasik[1], Agnieszka Grzybowska-Kowalczyk[1], Danuta Maslinska[2], Slawomir Maslinski[1,3]**

[1] Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland,

[2] Department of Experimental and Clinical Neuropathology, Medical Research Centre, Warsaw, Poland,

[3] Department of Pathophysiology, Medical University of Warsaw, Poland.

Aim: At a site of inflammation activated granulocytes generate large amounts of reactive oxygen species. A major oxidant is hypochloric acid (HOCl) which is generated by myeloperoxidase from hydrogen peroxide ($H_2O_2$) and chloride. HOCl reacts readily with amines to produce chloramines.

Histamine is stored in granules of mast cells and basophils at up to 100 mM concentration and released by inflammatory mediators. When these cells are activated at inflammatory sites, local concentration of histamine could be high enough to trap some of the HOCl released by activated neutrophils to produce histamine chloramine (HisCl). In this study we investigated the influence of HisCl on respiratory burst of human granulocytes (PMNs).

Materials & Methods: HisCl was prepared in our laboratory from NaOCl and histamine. Each preparation of HisCl was monitored by UV absorption (200-400nm) to confirm the presence of monochloramine (HisCl) and absence of dichloramine ($HisCl_2$).

Results: We found that HisCl inhibited luminol-dependent chemiluminescence (CL) of granulocytes in a dose-dependent manner ($10^{-6}$ – $10^{-3}$ M). Thioperamide H3/H4 reverse agonist completely blocked the inhibitory effect of HisCl on CL. Pyrilamine and cimetidine at all concentrations tested ($10^{-6}$ – $10^{-4}$M) were unable to antagonize the inhibitory effect of HisCl on oxidative burst of granulocytes.

Conclusion: The results of these studies suggest that HisCl inhibits oxygen radicals production by granulocytes and plays and important role in modulating of the inflammatory processes in vivo.

# CASPASE-MEDAITED ACTIVATION OF HISTIDINE DECARBOXYLASE IN A MOUSE MASTOCYTOMA, P-815

**Satoshi Tanaka**

Department of Immunobiology
School of Pharmaceutical Sciences, Mukogawa Women's University, Japan

[Background] Accumulating evidence suggests that histidine decarboxylase (HDC) undergoes post-translational processing in several kinds of cells, although it remains to be clarified how HDC is cleaved in these cells. Since the post-translational processing of HDC was found to affect its intracellular localization and enzymatic activity, the protease(s) involved in this process plays a critical role in regulation of histamine synthesis. Our purpose in this study is to identify the cleavage site and converting enzyme for HDC.

[Methods and Results] Alanine scanning mutagenesis was performed to determine the cleavage site of mouse HDC. Mutated and wild type HDC cDNA with an epitope tag was introduced into a mouse mastocytoma, P-815, using the lentiviral expression system. Treatment of the cells with butyrate was found to induce the cleavage of the full-length HDC (74-kDa) concomitantly with increase in enzymatic activity. The two DD motifs ($D^{517}D^{518}$, $D^{550}D^{551}$) were found to be crucial for generation of 55- and 60-kDa form. Both the limited cleavage and enzymatic activation of HDC upon treatment with butyrate were suppressed completely by a pan-caspase inhibitor, partially by specific inhibitors for caspase-8 and -9, but not by a caspase-3 inhibitor. Although treatment with butyrate augmented the enzymatic activity of caspase-3, -8, and -9, no apoptic cell death was observed.

[Conclusion] HDC is enzymatically activated by treatment of a mouse mastocytoma, P-815, with butyrate through its post-translational cleavage by caspases. Since the treatment of this cell line with butyrate was previously found to induce its granule maturation, activation of several caspases by butyrate may not induce apoptic cell death but promote the functional maturation.

# SIGNAL TRANSDUCTION MECHANISMS UNDERLYING THYMOSIN β4 MAST CELLS ACTIVATION

**Janina Wyczółkowska, Aurelia Walczak-Drzewiecka, Jarosław Dastych and Waldemar Wagner**

Center of Medical Biology, Polish Academy of Sciences, Lodz, Poland

Thymosin β4 (Tβ4) is a peptide, known to promote angiogenesis and wound healing. Because mast cells are engaged in these processes as well, we investigated the effect of Tβ4 on mast cells. Tβ4 at concentrations 0.2 to 2000 nM activated mast cells to release preformed mediators. Synthetic peptides derived from Tβ4 amino acid sequence were also capable to activate mast cells. While peptide AcSDKP matching 4 N-terminal amino acid residues of Tβ4 mediated low but detectable mediator release, peptides corresponding to sequences 16-38 and 17-23 of Tβ4 stimulated mast cells mediator release on the level equal or higher than that observed with native Tβ4. These observations and certain characteristics of Tβ4-mediated mast cell activation suggest that actin-binding motif LKKTET present in Tβ4 in position 17-22 might be engaged in this process. Tβ4 mast cell activation process was partially inhibited by pertussis toxin but was resistant to EGTA, and to high concentration of wortmannin - a PKB inhibitor. While resistance to wortmannin seems to contradict possible engagement of PINCH/ILK/PKB pathway in Tβ4-mediated mast cell exocytosis, the resistance to EGTA is consistent with possible engagement in this process G-actin known to directly interact with Tβ4 in calcium independent fashion. It is thus possible that Tβ4-mediated mast cell activation has mixed mechanism of action consisting of G-proteins dependent and non-dependent components. The latter might depend on direct interaction of Tβ4 with actin cytoskeleton that engages G-actin binding motif LKKTET.

In conclusion, Tβ4 previously identified as important mediator of tissue repair processes in human and in animals activated mast cells to release mediators that promote healing process in a process that demonstrated certain characteristics suggesting involvement of G-proteins and actin filaments.

# EFFECTS OF PACAP, VIP, AND SOME RELATED PEPTIDES, ON CYCLIC AMP GENERATING SYSTEM IN RAT NEURONAL AND ASTROCYTE CELL CULTURES

**Jóźwiak-Bębenista M., Bednarek K., Nowak J.Z.**

Department of Pharmacology, Medical University of Lodz, Poland

BACKGROUND: Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) belong to structurally related family of polypeptides, also comprising peptide histidine-isoleucine (PHI), peptide histidine-methionine (PHM), helodermin and secretin. PACAP and VIP are endogenous peptides, widely expressed in the central nervous system and peripheral organs of various vertebrates and characterized by rich profile of biological activity. Both peptides exert their actions via common receptors: VPAC1 and VPAC2, which bind VIP and PACAP with similar affinity. Additionally, PACAP also stimulates PAC1 type receptors with much higher affinity than VIP. The adenylyl cyclase (AC)/cyclic AMP (cAMP) is the main intracellular signal transduction pathway coupled with all mentioned types of receptors.

AIM: The present work is a continuation of our previous studies on the effects of various neuropeptides, including PACAP and VIP, on the AC→cAMP-linked signaling pathway, carried out on slices of rat cerebral cortex. In this study we have tested the ability of PACAP/VIP-family of peptides to stimulate cAMP generation in cultured astrocytes and neurons originating from rat cerebral cortex.

MATERIALS AND METHODS: The peptides used were: long PACAP form (PACAP38), mammalian VIP (mVIP), porcine and human PHI (pPHI and PHM), helodermin, secretin; we also tested some non-peptide agents (forskolin, isoprenaline, histamine). The biological material consisted of primary [3H]adenine-prelabeled neuronal cultures - originated from cerebral cortex of E16-E17 Wistar rat embryos, and astrocyte glial cultures - prepared from cerebral cortex of 1-day old Wistar rats.

RESULTS: All the neuropeptides disclosed strong and concentration (0.001-3 μM)-dependent stimulation of cAMP production in neuronal and glial cultures, displaying the following rank order of potency: PACAP38 > mVIP ≥ PHI/PHM ≈ helodermin > secretin. As expected, a β-adrenoceptor agonist isoprenaline (10 μM) and a direct activator of adenylyl cyclase forskolin (10 μM) also strongly stimulated cAMP generation in the two model systems, whereas histamine (100 μM) had no effect. The effects of PACAP, VIP and PHI on AC-driven signaling system in slices of rat cerebral cortex were weaker than those in rat primary cell cultures. Yet, comparing the two cell cultures, the examined peptides produced significantly larger cAMP responses in glial cells than in neuronal cultures.

CONCLUSUION: The obtained results showed that the rat cerebral cortex-derived neurons and glial cells respond well to PACAP, VIP, and some related peptides. Moreover, they express PAC1 and probably also VPAC type receptors linked to the activation of AC→cAMP signaling system. The largest cAMP responses observed in glial cells suggest that this cellular compartment is likely the main target for the studied peptides.

*Sponsored by grant 3P04C 076 25 (MNiI) and funds from Medical University of Lodz, Poland.*

# EFFECTS OF ADRENALINE ON CYCLIC AMP GENERATING SYSTEM AND EXPRESSION OF VEGF AND IL-8 IN HUMAN ENDOTHELIAL CELLS

**M.Namiecińska[1], A.Wiktorowska-Owczarek[2], J.Z.Nowak[1,2]**

[1] Center of Medical Biology, Polish Academy of Sciences, Lodz, Poland;
[2] Department of Pharmacology, Medical University of Lodz, Poland;

BACKGROUND and AIM. Several types of endothelial cells originating from blood vessels of different species, including human, are capable of synthesizing cyclic AMP generating system. Some authors have recently shown a link of the cyclic AMP pathway with the expression of factors regulating angiogenesis (eg. vascular endothelial growth factor, VEGF). Among stimulators of these systems are catecholamines, however published data do not precise the sequence of molecular events connecting catecholamines and VEGF. Thus, the aim of this work was to test major catecholamines (adrenaline, A: noradrenaline, NA; dopamine, DA; isoprenaline, ISO for their ability to stimulate synthesis of cyclic AMP, VEGF and IL-8 under various experimental conditions in cultured human microvascular endothelial cells (HMEC-1)[3/]. An additional aim of our work was to check whether A modulates the expression of VEGF and IL-8 in HMEC-1 cells. For comparative purposes, some experiments were also conducted on macrovascular endothelial cells originating from human umbilical vein (HUVEC).

MATERIALS AND METHODS: Cells used in experiments were seeded in 12-well plates at a density of approximately 250 000 cells/well. The formation of [$^3$H]cyclic AMP was studied in [$^3$H]adenine-prelabeled cells; double column-system was used for [$^3$H]cyclic nucleotide isolation. VEGF and IL-8 concentrations in cell culture media were determined by commercially available ELISA kits.

RESULTS: HMEC-1 cells only weakly synthesized cyclic AMP under basal non-stimulated conditions (experiments in the presence or absence of a PDE inhibitor, IBMX). A direct stimulator of adenylyl cyclase forskolin and adrenergic drugs, such as A, NA and ISO, strongly increased cyclic AMP accumulation in IBMX-pretreated HMEC-1 cells. Among tested catecholamines, A appeared to be the most powerful stimulator (at 100 μM - 8,43% of conversion); its effect was mimicked by ISO, while NA appeared to be clearly less potent, and DA practically inactive. The cyclic AMP effect of A was concentration-dependently antagonized by β-adrenoceptor blocker propranolol, and not affected by prazosin and yohimbine, α1- and α2-adrenoceptor selective antagonists, respectively. A did not significantly affect the ability of HMEC-1 cells to produce VEGF or IL-8, two major proangiogenic mediators, whereas – as expected – cobalt chloride, a hypoxia mimicking agent, did stimulate VEGF expression. Our preliminary findings coming from experiments carried out on macrovascular HUVEC cells showed that the A-evoked cyclic AMP responses in these cells were decisively smaller than those observed in HMEC-1 cells.

CONCLUSION: Adrenaline is a strong stimulator of cyclic AMP production in microvascular HMEC-1 cells, and a clearly weaker activator of this signaling system in macrovascular HUVEC cells. The amine does not affect levels of VEGF or IL-8 at least in HMEC-1 cells. The results will be discussed in terms of functional meaning of A effects in microvascular and macrovascular human endothelial cells.

*Supported by funds from Medical University of Lodz and by Center of Medical Biology, PAS, Lodz, Poland*

# CARDIAC EFFECTS OF THE ACTIVATION OF THE LOW AFFINITY STATE OF $\beta_1$-ADRENOCEPTORS IN PITHED RATS

**B. Malinowska[1], A. Zakrzeska[1], H. Kozłowska[1], E. Schlicker[2],**

[1]Department of Experimental Physiology, Medical University of Białystok, Poland,
[2]Department of Pharmacology and Toxicology, University of Bonn, Germany.

Activation of the low-affinity state of β1-adrenoceptors increases frequency and contractile force and hastens relaxation in isolated human and rat cardiac tissues in vitro. In addition, two agonists at this receptor, CGP 12177 and cyanopindolol, relax isolated rat and human vessels. We decided to determine the role of the low-affinity state of β1-adrenoceptors in the regulation of the cardiovascular function in vivo in pithed and vagotomized rats.

We found that CGP 12177 (0.1 – 100 nmol/kg) and cyanopindolol (1 – 1000 nmol/kg) increased in a dose-dependent manner heart rate (HR; by maximally 40 and 30% of the basal values) and myocardial contractility as reflected by the enhancement of the left ventricular systolic pressure (LVSP) by maximally 30 and 20% of the basal values and the rate of intraventricular pressure rise (+dP/dt-1max) by maximally 120 and 60% of the basal values, respectively. In addition, the rate of ventricular relaxation, reflected by the intraventricular pressure decline (-dP/dt-1max), was increased by maximally 80 and 50% of the basal values, respectively. The positive chronotropic effects of both agonists remained almost unchanged for 30 min after their administration, whereas their positive inotropic action (both LVSP and +dP/dt-1max) decreased by about 50% over the period of 30 min. All cardiac effects of CGP 12177 and cyanopindolol were diminished by 10 µmol/kg of the non-selective β-adrenoceptor antagonist bupranolol (which also antagonizes the low-affinity state of β1-adrenoceptors) and of CGP 20712A (known to block the low-affinity state of β1-adrenoceptors at high doses).

In contrast to the four cardiac parameters, simultaneous determination of two vascular parameters, diastolic blood pressure (DBP) and mesenteric blood flow (MBF), led us to a quite different conclusion. Thus, the highest doses of CGP 12177 and cyanopindolol increased DBP only by about 10% of the basal values. MBF was increased only by the highest doses of both agonists by about 31 and 13% of the basal values, respectively. None of the changes in vascular parameters elicited by the non-conventional β-adrenoceptor agonists was mediated by the low-affinity-state of β1-adrenoceptors since they were not affected by bupranolol or CGP 20712A (10 µmol/kg each).

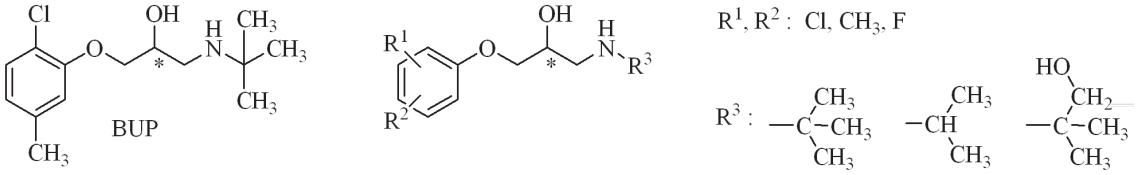
In conclusion, our results show that activation of the low-affinity state of β1-adrenoceptors by CGP 12177 and cyanopindolol in pithed rats causes a positive chronotropic, inotropic and lusitropic effect. By contrast, the vascular effects of CGP 12177 and cyanopindolol are not mediated by these receptors and have only marginal influence under in vivo conditions.

# BUPRANOLOL – AN OLD DRUG WITH NEW PERSPECTIVES

**D. Żelaszczyk[1], H. Kozłowska[2], U. Baranowska[2], K. Kieć-Kononowicz[1], B. Malinowska[2], E. Schlicker[3]**

[1] Department of Technology and Biotechnology of Drugs, Medical College of Jagiellonian University, Cracow, Poland
[2] Department of Experimental Physiology, Medical University of Białystok, Poland
[3] Department of Pharmacology and Toxicology, University of Bonn, Germany

Bupranolol (BUP) demonstrates antagonistic activity against all known subtypes of β-adrenoceptors (β-AR), including the low affinity state of β1-AR (also known as atypical β-adrenoceptors). It contains a chiral center and its side chain is similar to that of most other β-blockers, except that a tertiary butyl group replaces the more common isopropyl group.




Scheme 1.

The aim of our study was to synthesize racemic and enantiomeric analogues of bupranolol (the modifications showed on scheme 1) and to check which structural properties are responsible for its antagonistic effect at the low affinity state of ß1-adrenoceptors. For this purpose, the interaction of these compounds with CGP 12177 (which increases heart rate by activation of the low affinity state of β1-adrenoceptors) was determined in pithed and vagotomized rats. For comparison, the potencies of these drugs at the high affinity state of β1-adrenoceptors in the pithed rat (interaction with the cardiostimulatory effect of xamoterol) and their affinities at β1-adrenoceptor binding sites (inhibition of [3H]CGP 12177 binding studied in the presence of the β2-adrenoceptor antagonist ICI 118551) in rat brain cortex membranes were studied as well.

The following results were obtained:

| | Potency ($pK_B$) | | Affinity ($pK_i$) |
|---|---|---|---|
| | against CGP 12177 | against xamoterol | [$^3$H]CGP 12177 binding |
| (-)-BUP | 6.1 | 7.9 | 8.7 |
| (-)-GD-6 | 6.1 | 8.1 | 8.4 |
| (+)-GD-6 | 4.5 | < 6 | 6.9 |
| (-)-DZ-13 | 5.6 | 8.4 | 8.5 |
| (+)-DZ-13 | 4.7 | 6.2 | 6.7 |
| DZ-51 | 5.1 | 7.3 | 8.4 |

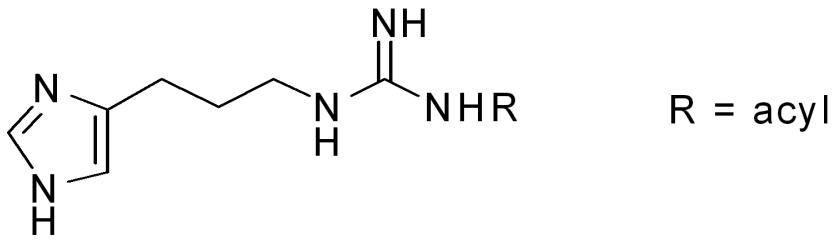
As a conclusion, the (-)-enantiomers of bupranolol derivatives show high affinity toward the b1-adrenoceptor and a potency comparable to that of BUP toward the low-affinity state of this receptor. The selected compouds show stereoselectivity at both states of the b1-AR.

# NEW $N^G$-ACYLATED IMIDAZOLYLPROPYLGUANIDINES AS POTENTIAL SELECTIVE HISTAMINE $H_2$-RECEPTOR AGONISTS

**Mirosław Łopuch[1], Anja Kraus[2a], Patrick Igel[2a], Sigurd Elz[2a], Roland Seifert[2b], Armin Buschauer[2a], Katarzyna Kieć-Kononowicz[1]**

[1] Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University, Kraków, Poland
[2] Institute of Pharmacy, University of Regensburg, Germany,
[a] Departments of Pharmaceutical/Medicinal Chemistry I and II, [b] Dept. Pharmacology and Toxicology

Histamine $H_1$- and $H_2$-receptors are postsynaptic G protein-coupled receptors (GPCRs). Activation of $H_1$-receptors coupling to $G_q$ is responsible for some symptoms of allergic reactions such as increased permeability of venules. In comparison to this $H_2$-receptor activation leads to stimulation of $G_s$ and is followed by increased heart rate and gastric acid secretion. $H_2$-receptor agonists might be used, for instance, in treatment of heart failure and acute myelogenous leukemia [1].
There are two chemical classes of $H_2$-receptor agonists: amines (e.g. dimaprit) and guanidines (e.g. arpromidine) [1]. Introduction of acyl group to guanidine-type compounds leads to potent $H_2$-receptor agonists with lower basicity and improved bioavailability [2].




R = acyl

Five novel $N^G$-acylated imidazolylpropylguanidines were synthesized and purified by HPLC. The structure elucidation was performed by means of $^1$H-NMR, $^{13}$C-NMR and HRMS.
Pharmacological data were obtained in the following experiments: test on the isolated ileum of the guinea pig ($H_1$-receptor antagonism), test on the isolated right atrium of the guinea pig ($H_2$-receptor agonism) and GTPase assay (agonism towards human $H_2$-receptor expressed in Sf9 cell membranes).

Conclusion: all compounds showed histamine $H_1$-receptor antagonism (maximal value of $pA_2 = pK_B = 7.22 \pm 0.05$) and histamine $H_2$-receptor agonism. In GTPase assay all compounds were partial agonists but much more potent than histamine (relative potencies 34.7-114.8).
This project was done at the University of Regensburg within the SOCRATES-Erasmus Programme and supported by the DFG (Research Training Group - Graduiertenkolleg GRK 760). Technical assistance of Mrs. K. Fisch by performing of GTPase assay is gratefully acknowledged.

*References:*
*1. Dove S., Elz S., Seifert R., Buschauer A.: Structure-Activity Relationships of Histamine H2 Receptor Ligands, Mini- Reviews in Medicinal Chemistry, 4: 941-954, 2004*
*2. Ghorai P.: Dissertation: Arpromidine-Related Acylguanidines: Synthesis and Structure-Activity Relationships of a New Class of Guanidine-Type Histamine H2Receptor Agonists with Reduced Basicity, Universität Regensburg, 2005, www.opus-bayern.de/uni-regensburg/volltexte/2006/561/*

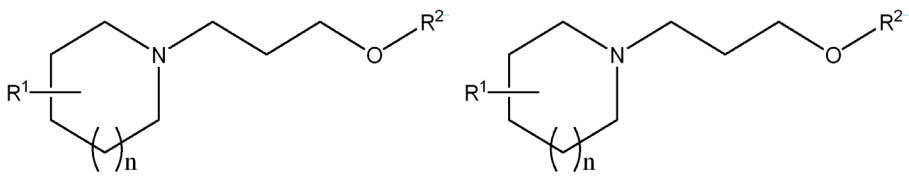
# LIPOPHILICITY PARAMETERS OF DIFFERENT PIPERIDINE DERIVATIVES AS HISTAMINE $H_3$ RECEPTOR ANTAGONISTS

**Kamil Kuder[1], Katarzyna Kieć- Kononowicz[1], Walter Schunack[2], Jean-Charles Schwartz[3], Xavier Ligneau[3], Holger Stark[4]**

[1] Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University, Kraków, Poland
[2] Institut für Pharmazie, Freie Universität Berlin, Germany
[3] Bioprojet Biotech, Saint Grégorie Cedex, France
[4] Institut für Pharmazeutische Chemie, ZAFES, Johann Wolfgang Universität, Biozentrum, Frankfurt am Main, Germany

Histamine $H^3$ receptors are constitutively active Gi-protein coupled receptors mostly expressed in central nervous system (CNS), and described as presynaptically located auto- as well as heteroreceptors. Blockade of this receptor leads to increased levels of histamine and modulated levels of many other neurotransmitters, e.g. ACh, NA, 5-HT. Therefore, histamine $H^3$ receptor antagonists may provide novel therapies for treating such CNS disorders like Alzheimer's disease, ADHD, dementia, Parkinson's disease, epilepsy, or obesity [1].

Antagonists could structurally be divided into two classes: imidazole, and non-imidazole derivatives. Based on our previous research we synthesized (methyl)piperidine, bipiperidine, and homopiperidine derivatives according the scheme:




$R^1$= piperidino, $CH_3$-, H-
$R^2$ = aryl, alkylaryl
n= 1, 2

In aim to evaluate the structure–bioactivity relationships we experimentally determined their lipophilicity parameters expressed by $R_{M0}$ values using planar RP-TLC method. The theoretical partition coefficient parameters (logP) were also calculated using different computer programs: HyperChem, PALLAS, CAChe Project Leader, SciLogP for Alchemy [2]. The novel compounds were evaluated for the histamine $H^3$ receptor activity in vitro in a binding assay for the human histamine $H^3$ receptor stably expressed in CHO-K1 cells. Compounds showed moderate to good histamine $H^3$ receptor affinities.

The correlation between parameters of lipophilicity ($R_{M0}$, logP) and binding properties ($K_i$ values) was determined.

*References:*

1 *Stark, H. et. al. Mini Rev. Med. Chem. 2004, 4, 965–977; Cowart, M. et. al. Mini Rev. Med. Chem. 2004, 4, 979–972*
2 *HyperChem 6.01 by Hypercube Inc., PALLAS 3.2.2.1 (demo version) by CompuDrug, CAChe Project Leader 6.1 by Fujitsu Ltd., SciLogP 3.0 for Alchemy (demo version) by SciVision*

# BENEFICIAL EFFECTS OF A PLANT HISTAMINASE IN A RAT MODEL OF SPLANCHNIC ARTERY OCCLUSION AND REPERFUSION.

**Emanuela Masini[1], Salvatore Cuzzocrea[2,3], Daniele Bani[4], Emanuela Mazzon[2,3], Carmelo Mujà[2], Rosanna Mastroianni[1], Francesca Fabrizi[1], Paola Pietrangeli[5], Lucia Marcocci[5], Bruno Mondovì[5], Pier Francesco Mannaioni[1] and Rodolfo Federico[6].**

[1] Department of Preclinical and Clinical Pharmacology, University of Florence; viale G.Pieraccini, 6; I-50139 Florence, Italy
[2] Institute of Pharmacology, University of Messina; Torre Biologica, Policlinico Universitario; I-98123 Messina, Italy.
[3] IRCCS Centro Neurolesi „Bonino-Pulejo", Messina, Italy.
[4] Department of Anatomy, Histology & Forensic Medicine, Section of Histology, University of Florence; viale G.Pieraccini, 6; I-50139 Florence, Italy
[5] 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, I-00185 Rome, Italy
[6] Department of Biology, 3rd University of Rome, Viale G. Marconi, 446, I-00146 Rome, Italy

Splanchnic artery occlusion (SAO) followed by reperfusion causes endothelial injury and inflammation which contribute to the pathophysiology of shock. We investigated the effects of pea seedling (Latyrus cicera) histaminase, known to afford protection against the deleterious effects of cardiac ischemia/reperfusion, given to rats subjected to SAO/reperfusion-induced splanchnic injury.

Histaminase (80 IU $kg^{-1}$, 15 min. before reperfusion) significantly reduced the drop of blood pressure and high mortality rate caused by SAO/reperfusion. Histaminase also reduced histopathological changes, leukocyte infiltration (myeloperoxidase) and expression of endothelial cell adhesion molecules in the ileum. Histaminase counteracted free radical-mediated tissue injury, as judged by significant decrease in the plasma and tissue levels of peroxidation and nitration products (oxidized rhodamine, malondialdehyde, nitrotyrosine), DNA damage markers (8-hydroxy-2'-deoxyguanosine, poly-ADP-ribosylated DNA) and consumption of tissue antioxidant enzymes (superoxide dismutase). As a result, histaminase led to a reduction of ileal cell apoptosis (caspase 3, TUNEL-positive cells).

These results show that histaminase exerts a clear-cut protective effect in SAO/reperfusion-induced splanchnic injury, likely due to oxidative catabolism of pro-inflammatory histamine as well as anti-oxidant effects resulting in hindrance of free radical-mediated tissue injury endothelial dysfunction and leukocyte recruitment. Thus, histaminase could be used therapeutically in intestinal ischemia.

# EFFECTS OF ANTIHISTAMINE TREATMENT ON ULCERATIVE COLITIS, BASED ON RAT MODEL*

**W. Agnieszka Fogel,**

Department of Hormone Biochemistry,
Chair of General Endocrinology,
Medical University of Lodz, Lodz, Poland

Histamine plays important roles in physiology and pathology of the mammalian gastrointestinal tract. Among others, it stimulates gastric acid secretion, influences gut motility and controls intestinal vasodilatation. Mast cell hyperplasia, i.e., an enhanced release of intestinal mast cell mediators and reduced histaminase activity, are characteristic features
of inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease. Experimental evidence will be provided to show that an administration of histamine receptor antagonists, as well as preparation of diamine oxidase, reduces the markers and signs of
inflammation and accelerates healing of damaged mucosa. The results will thoroughly be discussed in relation to the data, published so far on this issue, regarding clinical and experimental studies of IBD.

** supported by grant No 3 P05B 003 24 from Ministry of Science and Higher Education*

# EFFECTS OF MELATONIN ON ADRIAMYCIN-INDUCED CARDIOTOXICITY IN RATS

**K. Dąbrowska[1], M. Stuss[1], J. Gromadzińska[2], W. Wąsowicz[2], E. Sewerynek[1]**

[1] Department of Bone Metabolism; Chair of General Endocrinology, Medical University of Lodz, Poland;
[2] Department of Toxicology and Carcinogenesis, The Nofer Institute of Occupational Medicine, Lodz, Poland

Background: Adriamycin, an anthracycline antibiotic, is a potent, broad-spectrum chemotherapeutic agent that has been found to be effective in treatment in leukemias, lymphomas and a variety of solid tumours. However, its clinical usage is often limited by its side effects, especially cardiotoxicity. The induction of adriamycin-induced cardiotoxicity has been attributed to various mechanisms, oxygen radical-induced injury of membrane lipids being the most important one.
Melatonin (5-methoxy-N-acetyl-tryptamine), a pineal hormone, is a well-known antioxidant and free radical scavenger. This hormone has been found to be effective in protecting cells, tissues and organs against oxidative damage, induced by a variety of free radical generating agents and processes, including lipopolysaccharide, hydrogen peroxide, ischaemia-reperfusion and others. Our previous observations have shown that melatonin can scavenge free radicals not only directly but also indirectly, stimulating the production of many antioxidative enzymes, among which, GSH-Px is the most important one.

Objectives: The aim of the present study was to examine the effect of melatonin against adriamycin-induced cardiotoxicity in rats.

Materials and Methods. Wistar rats were divided into the following 3 maingroups: pinealectomized (PX), sham-operated (Sham-PX) and control animals (Intact). Each of the groups was divided into 4 Subgroups, injected with: 1 – saline, 2 – melatonin (MEL), 3 – adriamycin (ADR) and 4 – melatonin plus adriamycin (MEL + ADR). Pinealectomies were performed, according to the method of Kushak and Rodin. The rats were operated 2 months before the beginning of the treatment. Adriamycin was administered in a single dose (15 mg/kg) by intraperitoneal injection (i.p.), 1 hour after the fourth melatonin injection. Melatonin (5 mg/kg) was administered i.p. for 8 days (4 days before and 4 days after ADR injection). After 8 days of treatment, all the rats were killed by decapitation. Their hearts were removed, weighed, frozen and kept in -80°C till the biochemical measurements were performed.

Results: In the experiment, a decrease of heart mass was observed in all the examined groups after ADR + MEL and in the group of Intact animals after ADR. Lipid peroxidation increased statistically significantly after ADR in the groups of intact and sham-PX animals. Mel did not decrease ADR-induced LPO, either in intact or sham-PX rats.
The activity of GSH-Px, an antioxidative enzyme, in the heart increased significantly in all the examined groups after ADR injections.

Conclusion: Unexpectedly, in the present experiment, MEL did not protect against free radical damage, induced by ADR. These results may be explained by a possible strong effect of some endogenous antioxidative enzymes and agents, stimulated after the administration of ADR alone. That endogenous antioxidative system, including the activity of GSH-Px, as measured in the experiment, may have tried neutralising free radicals, especially during the first days of treatment.

*The study was supported by Grant No 502-11-293 of the Medical University of Łódź*

## LIST OF PARTICIPANTS

Urszula Baranowska
Department of Experimental Physiology, Medical University of Białystok, Poland

Agnieszka Cudnoch-Jędrzejewska
Department of Experimental and Clinical Physiology, Medical University of Warsaw, Poland.

Katarzyna Dąbrowska
Department of Bone Metabolism, Chair of General Endocrinology, Medical University of Lodz, Poland

W. Agnieszka Fogel
Department of Hormone Biochemistry, Chair of General Endocrinology, Medical University of Lodz, Lodz, Poland

Bartłomiej Grobelski
Department of Hormone Biochemistry, Chair of General Endocrinology, Medical University of Lodz, Lodz, Poland

Agnieszka Grzybowska-Kowalczyk
Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland

Grażyna Janikowska
Division of Analytical Chemistry, Medical University of Silesia, Katowice, Poland

Dariusz Jędrzejewski
University School of Medicine, Lublin, Poland

Jerzy Jochem
Department of Physiology, Medical University of Silesia, Zabrze, Poland

Marta Jóźwiak-Bębenista
Department of Pharmacology, Medical University of Lodz, Poland

Małgorzata Karbownik
Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Poland

Katarzyna Kieć-Kononowicz
Department of Technology and Biotechnology of Drugs, Medical College of Jagiellonian University, Cracow, Poland

Hanna Kozłowska
Department of Experimental Physiology, Medical University of Białystok, Poland

Kamil Kuder
Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University, Kraków, Poland

Mirosław Łopuch
Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University, Kraków, Poland

Barbara Malinowska
Department of Experimental Physiology, Medical University of Białystok, Poland

Urszula Mazurek
Division of Molecular Biology, Medical University of Silesia in Katowice, Poland

Magdalena Namiecińska
Center of Medical Biology, Polish Academy of Sciences, Lodz, Poland

Krystyna Sasiak
Ethical Commission for Animal's Research, Medical University of Lodz, Poland

## LIST OF PARTICIPANTS

Ewa Sewerynek
Department of Bone Metabolism, Chair of General Endocrinology, Medical University of Lodz, Poland

Barbara Skrzydło-Radomańska
Department of Gastroenterology, University School of Medicine, Lublin, Poland

Anna Stasiak
Department of Hormone Biochemistry, Chair of General Endocrinology, Medical University of Lodz, Poland

Grzegorz Szewczyk
Chair and Department of General & Experimental Pathology, Medical University of Warsaw, Poland

Dariusz Szukiewicz
Chair and Department of General & Experimental Pathology, Chair and Department of Obstetrics & Gynecology, 2nd Faculty of Medicine, Medical University of Warsaw, Poland

Aneta Wagner
Clinic of Alergology, Lodz, Poland

Waldemar Wagner
Center of Medical Biology, Polish Academy of Sciences, Lodz, Poland

Agnieszka Więcławek
Division of Molecular Biology, Medical University of Silesia, Katowice, Poland

Małgorzata Wojciechowska
Chair and Department of General & Experimental Pathology Medical University of Warsaw, Poland

Dorota Żelaszczyk
Department of Technology and Biotechnology of Drugs, Medical College of Jagiellonian University, Cracow, Poland

Krystyna Żwirska-Korczała
Department of Physiology, Medical University of Silesia, Zabrze, Poland

### Invited speakers:

Prof. Dr. Walter Schunack
Institut für Pharmazie, Freie Universität Berlin, Germany

Prof. Dr. Manfred Göthert
Institute of Pharmacology and Toxicology, University of Bonn, Germany

Prof. Dr. Andrzej Lewiński
Rector of Medical University of Lodz, Poland

Prof. Dr. Bruno Mondovĕ
Department of Biochemical Sciences “A. Rossi Fanelli” and C.N.R. Institute of Molecular Biology and Pathology, University of Rome “La Sapienza”, Rome, Italy

Prof. Dr. Satoshi Tanaka
Department of Immunobiology, School of Pharmaceutical Sciences, Mukogawa Women’s University, Japan

## Scientific & Organising Committee

W. Agnieszka Fogel
Barbara Skrzydło-Radomańska
Małgorzata Karbownik
Ewa Sewerynek
Krystyna Sasiak
Anna Stasiak
Dariusz Jędrzejewski
Bartłomiej Grobelski

## Organisers

The Polish Histamine Research Society
&
Department of Hormone Biochemistry
Medical University of Lodz

Supported by
the Ministry of Science and Higher Education