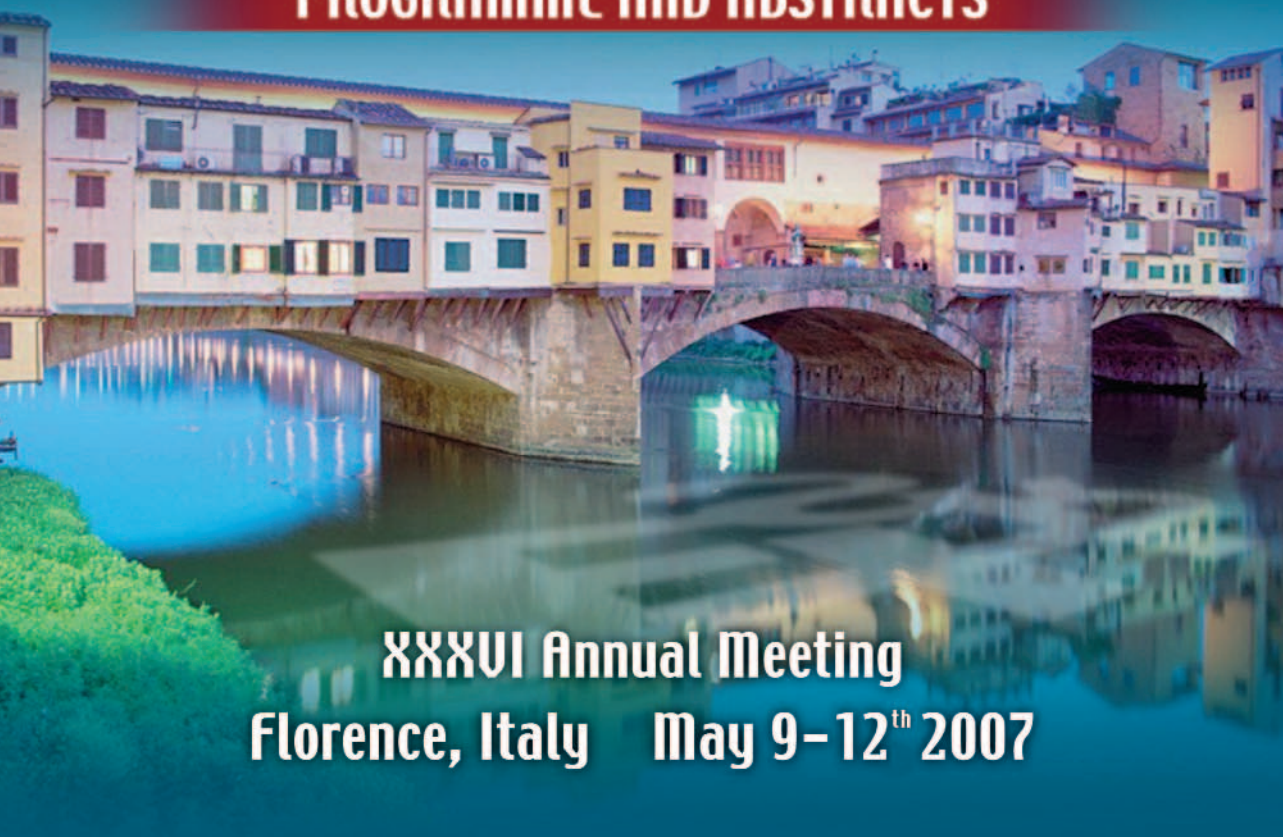
EUROPEAN HISTAMINE RESEARCH SOCIETY

EHRS

PROGRAMME AND ABSTRACTS



XXXVI Annual Meeting

Florence, Italy May 9-12th 2007

Contents

Sponsors

The Art Hancock Young Investigator Award

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Welcome from the Organizing Committee

Dear Histaminologists,

on behalf of the Organizing Committee, it is a great pleasure and honor to welcome you to the 36th Annual Meeting of the EHRS in Florence. It is the third time that Florence is the host of the meeting of this "Unique Society" and "unique" is Florence too, for its Renaissance art and Italian geniouses born here.

I also feel that Histamine research during the last ten years is living a New Renaissance, for the recent discovery of new classes of histamine receptors "and now they are four" and their involvement in important pathophysiological processes.

We offer you a very interesting program with plenary lectures, oral presentations and posters. Poster prizes will be awarded, as well as prizes for the best two oral presentations by Young Scientist will be revealed in the competitive "Art Hancock Young Investigator Award Symposium" sponsored by Abbott and Mrs Katrin Hancock.

Besides science, we will enjoiy social events in special places: the Opening Ceremony in Palazzo Vecchio, "Salone de' Cinquecento" the heart of Florence, the visit to Uffizi, Bargello and San Marco for the accompanying persons, and the excursion to Siena and Chianti.

The Symposium could not have been organized without the moral support of many colleagues and young collaborators and the financial support of some Institutions, Banks and Pharmaceutical Companies. The organizers express their thanks to all the participants who made this event happen and to the Sponsors for all support received.

On behalf of the organizing committee.

Emanuela Masini

Organizing Committee

Emanuela Masini "Chairperson"
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Madeleine Ennis "Past President EHRS"
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Jerzy Jochem
Erna Pap
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Holger Stark
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General Information

Congress Venue

Maglio Cloister - Chiostro del Maglio - Military Centre of Florentine Forensic Medicine
Via Venezia 5, Firenze.

Registration Desk

Registration desk will be open at the Maglio Cloister in the same time of the congress program.

Social Program

11th May 2007 - **Siena and Chianti**
The excursion leaves directly from the congress veue at 14:00 Return is prewied aroud 23:00.

12th May 2007 - **Palazzo Borghese**
Gala dinner takes place at 20:00 in this ancient palace in the center town.
Palazzo Borghese - Via Ghibellina, 110 - Firenze.

Presentation instructions

Poster Presentations

Authors should present their posters during the assigned session. The main point(s) from the poster will have to be presented in 1 min, followed by a 4 min discussion. The posters will be on display throughout the meeting. We encourage the presenters to stand by their posters during the interval and the poster session. The maximum poster size is 150 x 100 cm (h x w). All posters should have their corresponding code consisting of the letter "P" and a two-digit number (P__) in an A5 sized area (140 x 210 mm; h x w) on the top left corner of your poster. The code font should be Arial bold (or equal) and the size 240 pt (about 6 cm). Material to attach the posters will be provided. Posters which have made the final short-listing for the poster prizes will be revisited by the Poster Jury on Saturday afternoon. Prizes will be announced at the Farewell Dinner.

Oral Communications

Oral presentations should last no longer than 10 min and there will be 5 min for discussion.
The presentations should be in MS Power Point format (latest PPT version available: Microsoft Office 2003). A PC equipped with MS Powerpoint 2003 for Data Projection will be available in the Conference Venue. The projector is only PC and NOT Mac compatible. The presentation should be brought in a USB pen saver, saved in a file as: Name_of_presenter.ppt
It is advisable to have an extra copy of the presentation on a CD ROM. Please check the saved version on another computer beforehand. A common problem with Powerpoint files is that inserted images and animations have not been embedded in the file at all.
During the meeting you may preview your presentation on Wednesday 9 May 2007 at the Registration desk in the Maglio Cloister from 14.00-18.00 and during the following days.

Information

For full information about the museums, transportations, restaurants, please ask to the Registration Desk.

PROGRAMME OF THE XXXVI ANNUAL MEETING OF EHRS

Wednesday May 9th

15:00-18:00 **Registration** (Maglio Cloister, Via Venezia, 5)

16:00-18:00 **EHRS Council meeting** (Maglio Cloister, Via Venezia 5)

18:30-20:00 **Opening Ceremony**
(Palazzo Vecchio, Salone de' 500, Piazza della Signoria)
Welcome of the representative of the Municipality of Florence

G.F. Gensini	Dean of the Faculty of Medicine
A. Sydbom	EHRS President
E. Masini	Chairperson of the Congress

Opening Lecture **Cristina Acidini**
Polo Museale Fiorentino Superintendent

20:00-21:00 **Welcome cocktail** (Salone de' 500)

Thursday May 10th (Maglio Cloister, Via Venezia, 5)

8:00-13:00 **Registration**

8:30-9:45 **Opening Session**

E. Masini	Chairperson of the Congress
A. Santoro	Director of the Military Center of Florentine Forensic Medicine (Maglio Cloister)
A. Sydbom	EHRS President

Bursary Winners

Honorary Membership to:

C.R. Ganellin	laudation by Sir J. Black
W. Schunack	laudation by H. Stark
H. Timmerman	laudation by R. Leurs

9:45-10:45 **G.B. West Lecture by R. Leurs** (the Netherlands)
And then there were four...
Introduced by H. L. Haas (Germany)

10:45-11:00 **Coffee break**

11:00-12:00 **Symposium 1: Histamine and the Central Nervous System** (I)

Chairmen **P. Panula (Finland) and K. Yanai** (Japan).

11:00-11:15 (O1) O.A. Sergeeva, B.P. Klyuch, D. Vandael, **H.L Haas** (Germany).
Dopaminergic excitation of histaminergic tuberomamillary neurons.

11:15-11:30 (O2) A. Anaclet, R. Parmentier, G. Guidon, C. Buda, J.P. Sastre, H. Ohtsu, H.L. Haas, **J.S. Lin** (France, Japan, Germany).
Respective role of histamine and orexin neurons in sleep-wake control.

11:30-11:45 (O3) **P. Giannoni**, M.B. Passani, D. Nosi, A.D. Medhurst, P. Chazot, F. Shenton, P. Blandina (Italy, UK).
Detection of functional heterogeneity of histaminergic neurons in response to GSK189254, a novel H_3 receptor antagonist.

11:45-12:00 (O4) ***P.L. Chazot***, F.C. Shenton, H. Waldvogel, D. Grandi, G. Morini (UK, Italy, New Zealand).
The H_4 histamine receptor is expressed in both the human CNS an rodent PNS.

12:00-13:00 **Symposium 2: Histamine and the Central Nervous System** (II)

Chairmen **A. Medhurst** (UK) and **J.S. Lin** (France).

12:00-12:15 (O5) **K. Yanai**, H. Dai, E. Sakurai, T. Watanabe (Japan).
The roles of histamine receptors on cognition.

12:15-12:30 (O6) **A.D. Medhurst** (UK).
Assessing the potential of novel histamine H3 receptor antagonists for the treatment of multiple CNS disorders.

12:30-12:45 (O7) B. Tang, J. Zhang, H-Z. Li, J-N. Zhu, **J-J. Wang** (China).
Excitatory effect of histamine on neuronal activity of rat cerebellar fastigial nucleus in vitro.

12:45-13:00 (O8) **P. Panula**, K. Karlstedt, M. Lintunen (Finland).
On the role of histamine in ethanol preference.

13:00-14:00 **Lunch**

14:00-15:30 **Poster session 1 with soft-drinks and coffee**
(in parallel with poster session 2).

Chairpersons **O. Selbach** (Germany) **and M. Carman-Krzan** (Slovenia).

Thursday May 10th (Maglio Cloister, Via Venezia, 5)

P1: **D. Szukiewicz** (Poland).
Human β-defensin 3 expression and mean histamine concentration-human placental tissue study.

P2: **D. Szukiewicz** , G. Szewczyk, M. Pyzlak, J. Klimkiewicz, D. Maslinska (Poland).
Increased production of human β-defensin 3 (hBD-3) by human amniotic epithelial cells (HAEC) after activation of toll-like receptor 4 (TLR4) in chorioamnionitis.

P3: ***G. Szewczyk***, M. Pyzlak, W. Miertka, J. Klimkiewicz, D. Szukiewicz (Poland).
Does histamine influence differentiation of trophoblast in preeclampsia?

P4: **E. Pap**, E. Pallinger, A. Kiss, E. Buzas, A. Falus (Hungary).
Microvesicles: a new immunological communication pathway between the fetus and the mother. Studies in asthmatic and healthy pregnancies.

P5: **P. Pocza**, K. Pocza, A. Falus, Z. Darvas (Hungary).
Histamine increase the invasive potential of human melanoma cells through H_1 and H_2 receptors.

P6: S. Nimmergeers, **J. Van de Voorde** (Belgium).
Characterization of the effect of histamine in mice corpus cavernosum.

P7: **Z. Darvas**, K. Boér, P. Pocza, E. Helinger, A. Helinger, N. Kiséry, V. Szente, A. Falus (Hungary).
Down regulation of histamine receptors in human colon cancer.

P8: **S. Sprogar**, I. Krinar, M. Drevensek, G. Drevensek (Slovenia).
Famotidine, a H_2 receptor antagonist, decreases the late phase of orthodon tic tooth movement in rats.

P9: I. Krinar, **S. Sprogar**, M. Drevensek, G. Drevensek (Slovenia).
Cetirizine, H_1 receptor antagonist, decreases the first stage of orthodontic tooth movement in rats.

P10: **D. Maslinska**, M. Maslinska, J. Opertowska, M. Wojciechowska, S. Maslinki (Poland).
Histamine releasing factor (HRF) in pannus of joints affected by rheumatoid arthritis.

P11: A. Grzybowska-Kowalczyk, **D. Maslinska** , M. Wojciechowska, E. Wojtecka-Lukasik, S. Maslinski (Poland).
Expression of histamine H_4 receptor in human osteoarthritic synovial tissue.

- **P12:** **J. Jochem**, E. Olearska (Poland).
Cardiovascular reactivity to histamine in a high-sodium intake model of pree clampsia in rats.

- **P13:** **A. Molina-Hernández**, I. Velasco (Mexico).
Effect of histamine on cell proliferation, apoptotic death and cell differentiation in cultured cortical neural stem cells, on the way to study the histaminergic role during cerebral cortex development.

14:00-15:30 **Poster session 2 with soft-drinks and coffee**
(in parallel with poster session 1).

Chairpersons **E. Tiligada** (Greece) and **E. Pap** (Hungary).

- **P14:** G.P. Cricco, N.A. Mohamad, L.A. Sambuco, F. Genre, M. Croci, A.S. Gutiérrez **V.A. Medina**, R. M. Bergoc, E.S. Rivera, G.A Martín (Argentina).
Histamine regulates pancreatic carcinoma cell growth through H_1, H_2, H_3 and H_4 receptors.

- **P15:** **K. Cerne**, T. Irman-Florjanc, M. Krzan (Slovenia).
Histamine uptake into human vascular endothelial cells and influence of some antidepressant drugs.

- **P16:** **T. Ishizuka**, K. Hatano, A. Yamatodani (Germany and Japan).
The histaminergic system is a promising target for the treatment of leptin resistant obesity.

- **P17:** **O. Artyomova**, D.A. Miskevich (Belarus).
The free histidine pool formation and it's relationship with plasma nitric oxide level under chronic alcohol intoxication.

- **P18:** **S. Rajtar**, T. Irman Florjanc (Slovenia).
Influence of amitriptyline on DAO and HNMT mRNA expression in different guinea pig tissues and on DAO release in guinea pig.

- **P19:** **O. Selbach**, J. H. Stehle, H.L. Haas (Germany).
Histaminergic and orexinergic interference with clock gene controls hippocampal synaptic plasticity.

- **P20:** W.A. Fogel, **J. Jochem**, A. Lewinski, M. Maksymowicz (Poland).
Toward regulation of food intake in portocavally shunted rats.

Thursday May 10th (Maglio Cloister, Via Venezia, 5)

P21: **J. Jochem**, T. Irman Florjanc, K. Zwirska-Korczala, A. Niwecka, R. Rybczyk (Poland and Slovenia).
Central serotonin-induced pressor effect in rats is mediated via the histaminergic system.

P22: K. Ales, B. Wraber, **M. Lipnik-Stangelj** (Slovenia).
The synergistic effect of histamine and IL-6 on NGF secretion from cultured a strocytes is evoked by histamine stimulation of IL-6 secretion via PKC-MAPK signalling pathway.

P23: **K.D. Kyriakidis**, M.C. Theodorou, P.S. Filippou, E. Tiligada, D. A. Kyriakidis (Greece).
Histamine effect on the expression of AtoS-AtoC two component system in Escherichia coli.

P24: G. Garcia-Faroldi, F. Correa-Fiz, H. Abrighach, J.L. Urdiales, F. Sanchez-Jimenéz, I. **Fajardo Paredes** (Spain).
Role of polyamines in the expression of histidine decarboxylase and histamine synthesis during mouse mast cell differentiation.

P25: **D. Vandael**, B. P. Klyuch, H.L. Haas, O.A. Sergeeva (Germany).
Excitation of histaminergic neurons by thyrotropin-releasing hormone.

P26: V. Nosalova, K. Drabilova, V. Jancinova, T. Macickova, **R. Nosal**, J. Pecivova, M. Petrikova, R. Sotnikova (Slovak Republic).
Effect of H_1 antihistamines in the model of mesenteric ischaemia/reperfusion.

15:30-16:30 **Symposium 3: Histamine receptors**

Chairpersons **G. Coruzzi (Italy) and H. Timmerman** (the Netherlands).

15:30-15:45 (O9) **R.G. Booth**, L. Fang, A. Wilczynski, S. Sivendren, Z. Sun, M. Bruysters, K. Sansul, R. Leurs (USA and The Netherlands).
Molecular determinants of ligand-directed Gq versus Gs signaling for the histamine H_1 receptor.

15:45-16:00 (O10) **G. Morini**, D. Grandi, G. Becchi, F. Shenton, P. Chazot (Italy and UK).
Histamine H_3 and H_4 receptors are expressed on distinct endocrine cell types in the rat fundic mucosa.

16:00-16:15 (O11) **H.D. Lim**, P. Sadek, A. Jongejan, E. Haaksma, I. de Esch, R. Leurs (The Netherlands).
Histamine H_4 receptor species variants and the molecular basis for their pharmacological differences.

16:15-16:30 (O12) **K. Sansuk**, P. Hensbergen, C. Balog, R. Bakker, R. Leurs (The Netherlands).
MS analysis of human histamine H1 receptors.

16:30-18:00 **Symposium 4: Histamine and Immune System**

Chairmen **A. Falus** (Hungary) **and F. Die**l (Germany).

16:30-16:45 (O13) **L.J. Kay**, P.T. Peachell (UK).
Effects of MAPK inhibitors on histamine and eicosanoid generation from human lung mast cells.

16:45-17:00 (O14) **H.K. Takahashi**, T. Watanabe, M. Nishibori (Japan).
Cimetidine induces IL-18 production through H2-agonist activity in monocytes.

17:00-17:15 (O15) **R. Khanferyan**, A. Andreyanova, D. Lesik, N. Milchenko (Russia).
Histamine and histamine receptor antagonists as an IgE-regulatory factors.

17:15-17:30 (O16) **M. Fisher**, H. Borck, J. Flynn, C. Krieg, F. Diel (Germany)
Ex vivo-assessment of allergens includes the histamine liberation test (HLT).

17:30-17:45 (O17) **I. Michel**, H. Borck, F. Diel (Germany).
Histamine receptor H4R-selective ligands influence the STAT6 Transcription Activation Domaine (TAD) and the DNA-binding.

17:45-18:00 (O18) **N. Hiedayati**, S. Liu, M. Ogasawara, **K. Maeyama** (Japan).
Effects of c-kit receptor mutation on the maturation and the function of rat peritoneal mast cells: using heterozygous Ws/+ rats.

Friday May 11th (Maglio Cloister, Via Venezia, 5)

8:30-9:30 **Invited lecture**
A. Parini (France).
Oxidative stress and monoamine oxidases: from basic studies to novel therapeutical interventions.

Introduced by B. Mondovì (Italy).

9:30-10:30 **Symposium 5: Physiological and pharmacological role of monoamine oxidases**

Chairmen **L. Raimondi** (Italy) and **H. Schwelberger** (Austria).

Friday May 11th (Maglio Cloister, Via Venezia, 5)

9:30-9:45 (O19) **S. Cuzzocrea**, D. Bani, E. Mazzon, C. Mujà, R. Mastroianni, F. Fabrizi, B. Mondovì, P.F. Mannaioni, R. Federico, E. Masini (Italy).
Beneficial effects of a plant histaminase in a rat model of splanchnic artery occlusion and reperfusion.

9:45-10:00 (O20) **H.G Schwelberger**, J. Feurle (Austria).
Characterization of human plasma amine oxidase.

10:00-10:15 (O21) P. Pietrangeli, L. Marcocci, E. Masini, R. Federico, **B. Mondovì** (Italy).
Possible pharmacological applications of copper amine oxidases.

10:15-10:30 (O22) **C. Alfarano**, L. Fracassini, B. Romagnani, R. Pirisino, L. Raimondi (Italy).
Methylamine, an endogenous regulator of adipocyte nitric oxide synthase activity.

10:30-10:45 **Coffee break**

10:45 -11:45 **Poster session 3, with soft drinks and coffee**
(in parallel with poster session 4).

Chairmen **H. Stark** (Germany) and **J. Jochem** (Poland).

P27: **E. Passante**, C. Ehrhardt, H. Sheridan, N. Frankish (Ireland).
In vitro inhibition of rat basophilic leukaemia mast cell (RBL-2H3) degranulation by novel indane compounds.

P28: **J. Sainte-Laudy**, P.F. Mannaioni, Ph. Belon (France and Italy).
Confirmation of biological effects of ultramolecular dilutions. Effects of ultramolecular concentrations of histamine, 4-methyl-histamine and of adrenaline on the activation of human basophils.

P29: **J. Ramos-Jiménez**, L-E. Soria-Jasso, J. Camacho, J.A. Arias-Montano, (Mexico).
Histamine augments β_2-adrenoceptor-induced cyclic AMP accumulation in human prostate cancer cells DU-145 independently of known histamine receptors.

P30: E. Melgarejo, **M. A. Medina**, F. Sanchez-Jiménez, J. L. Urdiales (Spain).
Epigallocatechin-3-gallate inhibits key features of mast cells related with inflammatory responses.

P31: **V. Laszlo**, I. Jelinek, R. Jager, E. Pallinger, T. Hieronymus, R. L. Thurmond, A. Falus (Hungary, Germany and USA).
H_4 Receptor mediated effects of histamine on mouse dendritic cells.

■ **P32:** **E. Pallinger**, Zs. Horvath, E.I. Buzas, H. Hegyesi, I. Jelinek, R.L. Thurmond, A. Falus (Hungary and USA).
The lack of H_4 receptor has a significant impact on the T cell development of H4R-KO mice.

■ **P33:** K. Isensee, M. Amon, B. Sasse, X. Ligneau, J.C. Schwartz, **H. Stark** (Germany and France).
Novel potent dual histamine H_1/H_3 receptor antagonists.

■ **P34:** **K. Kuder**, L. Lazewska, W. Schunak, H. Stark, X. Ligneau, J.C. Schwartz, K. Kiec-Kononowiciz (Poland, Germany and France).
Comparison of piperidine vs. piperazine derivatives as histamine H_3 receptor antagonists.

■ **P35:** C. Marzocca, **Vannacci A.**, Nistri S., Giannini L., Bani D., Gori A.M., Abbate R., Gensini G.F., Manoni M., Ceccarelli M., Mannaioni P.F., Masini E. (Italy).
Antiinflammatory effects of sulfated heparin-like semi-synthetic derivatives in carrageenan-induced model of inflammation in rat.

■ **P36:** **M. Adami**, E. Guaita, I.J.P de Esch, R. Leurs, H. Stark, G. Coruzzi (Italy, The Netherlands and Germany).
Gastric effects of the highly selective histamine H_3 receptor agonist methimepip.

■ **P37:** **G. Coruzzi**, M. Adami, E. Guaita, I.J.P. de Esch, R. Leurs (Italy and The Nethelands).
Antiinflammatory, analgesic and gastric effects of the novel and selective histamine H_4-receptor antagonists VUF10214 and VUF10148.

■ **P38:** **C.B. Collins**, A.W. Baird, D.P. Campion (Ireland).
Coupling mRNA expression to functional assessment of histamine receptor activity in the avian intestine.

■ **10:45-11:50** **Poster session 4, with soft drinks and coffee**
(in parallel with poster session 3).

Chairpersons **M.B. Passani** (Italy) and **E-S Assem** (UK).

■ **P39:** **M. Smith**, R.H. Garrett (USA).
Keyword-based, Boolean-logic driven data mining discloses correlations between enigmatic idiopathic conditions, occlusive vascular diseases such as tropical endomyocardial fibrosis (EMF) with unexplained eosinophilia, and "histamine dysmetabolism".

Friday May 11th (Maglio Cloister, Via Venezia, 5)

P40: R. Jarisch, K. Moritz, P. Moser, **W. Hemmer** (Austria).
Histamine in red wine: no correlation between histamine level and wine quality.

P41: **E. Benetti**, A.C. Rosa, M. Gallicchio, R. Fantozzi (Italy).
Substance P-induced cyclooxygenase-2 expression in polymorphonuclear cells.

P42: E. Wojtecka, A. Grzybowska-Kowalczyk, D. Maslinska, **D. Szukiewicz**, S. Maslinski (Poland).
Effect of histamine chloramine on luminol-dependent chemiluminescence of granulocytes.

P43: M. Fischer, C. Krieg, **F. Diel** (Germany).
In vitro-tests with human blood cells are a practical tool in the immunological risk assessment of chemicals.

P44: C. Gigante Perez, R. Khanferyan, **F. Diel** (Germany and Russia).
Music and Histamine.

P45: A. Steneberg, **F. Diel** (Germany).
Histamine intolerance-diagnosis and therapy.

P46: **B. Giera**, J. Kressel, E.G. Hahn, M. Raithel (Germany).
Symptoms in double blind placebo controlled histamine provocation.

P47: **B. Giera**, J. Kressel, E.G. Hahn, M. Raithel (Germany).
Plasma histamine levels in double blind placebo controlled histamine provocation.

P48: **S. Mayr**, A. Behnecke, O. Wendler, M. Raithel, H. Iro (Germany).
Evaluation of ECP release of intact tissue biopsies from patients with nasal polyps.

P49: **I. Ungvári**, G.T. Kozma, G. Tolgyesi, G. Losonczy, M. Keszei, Z. Komlosi, A. Falus, C. Szalai (Hungary).
Reduced asthma symptoms and altered gene expression profile in histamine deficient mice.

P50: **A. Vannacci**, F. Lapi, M. Moschini, E. Cecchi, M. di Pirro, G. Banchelli, A. Mugelli (Italy).
The Tuscan Centre for Pharmacovigilance: a regional centre for the diagnosis, management and prevention of Adverse Drug Reactions.
The case of anti-histamines.

■ **P51:** B.F. Gibbs, K. Papenfuss, **F.H. Falcone** (UK).
A new two-step procedure for the purification human peripheral blood basophils to near homogeneity.

■ **11:50-13:05** **Symposium 6: Histamine: a mediator for all seasons**
In honour of Prof. Pier Francesco Mannaioni

Chairpersons **R. Fantozzi** (Italy) and **M. Ennis** (UK).

■ **11:50-12:05 (O23)** **C. Mariottini**, S. Fossati, G. Mannaioni, G. Bongers, R. Leurs, A. Chiarugi, P. Blandina, M.B. Passani (Italy and The Netherlands).
Selective H_3 receptor isoforms activate Akt and ERK in rat cortical neurons.

■ **12:05-12:20 (O24)** **Y. Jelinek**, V. Laszlo, E. Buzas, E. Pallinger, B. Hangya, Z. Horvath, A. Falus (Hungary).
Increased antigen presentation and Th1-polarization in genetically histamine-free mice.

■ **12:20-12:35 (O25)** **B.F. Gibbs** (UK and Germany).
Differential regulation of IgE-mediated histamine release in human basophils by SHIP-1 and syk.

■ **12:35-12:50 (O26)** **S. Nistri**, L. Cinci, A.M. Perna, E. Masini, D. Bani (Italy).
Mast cell inhibition and reduced histamine-related ventricular arrhythmias in a swine model of acute myocardial infarction upon therapeutic administration of the cardiotropic hormone relaxin.

■ **12:50-13:05 (O27)** **R. Fantozzi** (Italy).
Pathophysiological modulation of histamine release: a correlation between in vitro and in vivo data.

■ **13:05-14:00** **Lunch**

■ **14:05 -22:00** **Social programme for all participants**

■ **14:10-19:00** **Excursion to Siena**

■ **19:00-22:00** **Dinner in Chianti** (Fattoria di Montepaldi) with music.

Saturday May 12th (Maglio Cloister, Via Venezia, 5)

8:30-9:30 **Invited Lecture**
J. Ring (Germany).
Anaphylaxis: pathophysiology and management.

Introduced by M. Ennis (UK).

9:30-10:50 **The Art A. Hancock Young Investigator Award Symposium**

9:30-9:35 **Introduced by L. Black** (USA).

Chairmen **B. Gibbs** (UK and Germany) and **L. Black** (USA).

9:35-9:50 (O28) **L. Giannini**, E. Masini, D. Bani, C. Uliva, C. Marzocca, S.A.A. Cohmair, C.S. Erzurum, M. Ndengele, D. Salvemini (Italy and USA).
Ceramide-driven oxidative/nitrative stress: a key pathway to asthma.

9:50-10:05 (O29) Z. Horváth, E. Pallinger, G. Horvath, A. Falus (Hungary).
Hematopoietic proliferation and IL-3 signaling are impaired in histidine decarboxylase knock out (HDC-ko) mice.

10:05-10:20 (O30) **F. Shenton**, R. van Rijn, R. Bakker, R. Leurs, P. Chazot (UK and The Netherlands).
Histamine H_4 receptor isoform hetero-oligomerisation and cell surface expression in vitro.

10:20-10:35 (O31) **R. Parmentier**, C. Anaclet, H. Ohtsu, M. William, J.S. Lin (France, Japan and USA).
The brain H_3-receptor as a novel therapeutic target for vigilance and sleep-wake disorders.

10:35 -10:50 (O32) **D. Dijkstra**, R. Leurs, P. Chazot, F.C. Shenton, H. Stark (Germany, the Netherlands and UK).
Histamine downregulates CCL2 production by monocytes and monocyte-derived inflammatory dendritic epidermal cells (IDEC) via the histamine H_4 receptor (H4R).

10:50-11:15 **Coffee break**

11:15-12:15 **Symposium 7: Histaminergic new ligands**

Chairpersons **W. Schunak** (Germany) and **M. Garbarg** (UK).

11:15-11:30 (O33) **J.D. Venable** (USA).
Synthesis and SAR of heterocyclic piperazines as potent histamine H_4 receptor ligands.

11:30-11:45 (O34) **N.I. Carruthers** (USA).
Exploration of structure activity relationships for dual H_3 antagonists/ serotonin transporter reuptake inhibitors.

11:45-12:00 (O35) **M. Cowart**, L. Black, H. Liu, C. Zhao, M. Strakhova, G. Diaz, T.A. Esbenshade, J. Brioni (USA).
Optimization of H_3 antagonist series for H_3 selectivity over the hERG channel.

12:00-12:15 (O36) *E. Zampeli*, R.L. Thurmond, E. Tiligada (Greece and USA).
Effect of H_4R antagonist JNJ7777120 on the histamine content in ratconjunctiva: a preliminary in vivo study.

12:15-13:00 **Symposium 8: Histamine: cell growth and differentation**

Chairpersons **F. Pearce** (UK) and **P.F. Mannaioni** (Italy).

12:15-12:30 (O37) **Z. Pos**, Z. Wiener, P. Pocza, M. Racz, H. Hegyesi, A. Falus (Hungary).
Histamine interferes with the insulin-like growth factor 2 receptor (Igf2R)-fibulin-5 (Fbln5) axis via H_1R in mouse melanoma.

12:30-12:45 (O38) **M. Carman-Krzan**, D.M. Juric (Slovenia).
Regulation of neurotrophin synthesis in astrocytes by histamine and other monoamines.

12.:45-13:00 (O39) **V.A. Medina**, E. Melgarejo, E.J.V. Crescenti, N. Massari, M.A. Nunez, G.P. Cricco, G.A. Martin, R. Bergoc, F. Sanchez-Jimenez, E. Rivera (Argentina and Spain).
Histamine pivotal role in regulating mammary carcinogenesis.

13:00-14:00 **Lunch**

14:00-15:30 **Symposium 9: Histamine and diseases**

Chairpersons **W. Lorenz** (Germany) and **G. Sturman** (UK).

14:00-14:15 (O40) **J. Kralova**, R. Nosal, K. Drabikova, V. Jancinova, P. Denev, A. Moravcova, L. Kubala, M. Ciz, A. Lojek (Czech Republic, Slovak Republic and Bulgaria).
Comparative investigations of the influence of H1-antihistamines on the generation of the reactive oxygen species by phagocytes.

14:15-14:30 (O41) **E-S. K. Assem**, K.H. Peh, B. Y. Wan, S. Manaviazar, M.A. Walters, J. H. George, K.J. Hale (UK).
Pharmacological actions of a new synthetic cyclodepsipeptide, the A83586C-citropeptin hybrid, v. complement C5a.

Saturday May 12th (Maglio Cloister, Via Venezia, 5)

14:30-14:45 (O42) **T. Sakata**, H. Yoshimatsu, T. Masaki (Japan).
Mastication and oral load of L-histidine induce efficient reduction of visceral adiposity through activation of histamine neurons.

14:45-15:00 (O43) **J. Sainte-Laudy**, M. Day, F. Machavoine, E. Schneider, A.F. Berton (France).
Comparison of the effect of 4-methyl histamine on human and mouse activated basophils.

15:00-15:15 (O44) **D. Laroche**, S. Lammens, C. Lefrancois, B. Plaud (France).
Leukotriene measurements for the diagnosis of peranaesthetic allergic events.

15:15-15:30 (O45) M. Hatano, C. Matsushita, M. Shahriar, Y. Kitamura, H. Mizuguchi, N. Takeda, **H. Fukui** (Japan).
Suppression of histamine H_1 receptor mRNA elevation in nasal mucosa of allergy model rats by prolonged pretreatment of antihistamines.

15:30-16:30 **Final poster viewing and poster evaluation committee meeting, with coffee and soft drink**

16:30-18:00 **General Assembly of EHRS members**

20:00-23:00 **Palazzo Borghese** (Via Ghibellina, 110)
Farewell Dinner.

Invited lectures

[1] L

And then there were four...

Rob Leurs
Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, de Boelelaan 1083, 1081 HV Amsterdam, the Netherlands.

Until 2000 histamine has been thought to act via three different G-protein coupled receptors. All three receptors have attracted considerable interest as therapeutic targets. Antagonists for the H_1-(e.g. fexofenadine in Allegra® or l-cetirizine in Xyzal®) or H_2 receptor (e.g. cimetidine in Tagamet® or ranitidine in Zantac®) have been successfully used for many years for the treatment of allergic conditions and gastric ulcers, respectively. Moreover, currently a lot of attention in the pharmaceutical industry is directed towards the therapeutic use of H_3 antagonist for e.g cognitive disorders and obesity.
And then there were four……Following the sequencing of the human genome, data mining efforts have revealed the existence of a new histamine receptor with high expression levels in mast cells and leukocytes. Using the sequence information of the human H3 receptor, several groups independently identified a homologous GPCR sequence in the human genome sequence databases. The 390 amino acid protein sequence is encoded by 3 different exons encompassing respectively amino acids 1 to 64, 65 to 119 and 120 to 390. The new GPCR is expressed predominantly in bone marrow, eosinophils and mast cells and the histamine H_4 receptor shows a clearly distinct pharmacological profile. In our laboratory we recently have developed various new H_4 receptor ligands and we try to rationalize their interactions with the receptor proteins. Moreover, several of the new compounds are used as pharmacological tools to delineate the role of the H4 receptor in (patho)-physiology.

[2] L

Oxidative stress and monoamine oxidases: from basic studies to novel therapeutical interventions

Angelo Parini
INSERM U858, Institut de Médecine Moléculaire de Rangueil-I2MR, Toulouse, France.

Biogenic amines, including catecholamine and serotonin, regulate a variety of cell functions through the interaction of G-coupled membrane receptors. During the last years, we described a novel mechanism of action of dopamine and serotonin that occurs independently of membrane receptor stimulation and requires hydrogen peroxide generation by monoamine oxidases (MAO).
Using different models of renal and cardiac cells we showed that, in addition to the classical receptor-dependent effects, dopamine and serotonin induces cell proliferation and hypertrophy by a mechanism independent of receptor activation. At higher concentrations (up to 10 μM), dopamine and serotonin cause cell apoptosis by sequential i) increase in the ratio of Bax/Bcl2 proteins, ii) mitochondrial cytochrome c release, iii) caspase 3 activation and iv) DNA fragmentation. Both proliferative and apoptotic effects of dopamine and serotonin were not inhibited by specific receptor antagonists but were prevented by amine transporter inhibitors, the irreversible MAO inhibitor pargyline and the antioxidant N-acetylcysteine. These data show that dopamine and serotonin induces cell proliferation, hypertrophy and apoptosis by a receptor-independent mechanism requiring amine internalisation into the cells, their degradation by MAOs and hydrogen peroxide production.
Based on these findings, we next investigated the potential role of hydrogen peroxide generated by MAOs on cell death in vivo. Our results showed that MAO inhibition largely reduced renal and myocardial damage induced by ischemia/reperfusion in rat. The protective effects of MAOs inhibitors were associated with the prevention of post-ischemic oxidative stress, increase in ceramide accumulation, neutrophil infiltration and mitochondrial-dependent cell death.
In conclusion, our results show the key role of H_2O_2 produced by MAOs in mediating cell effects of biogenic amines and propose these enzymes as a pharmacological target for prevention of organ damages.

[3] L

Anaphylaxis: pathophysiology and management

Johannes Ring
Klinik und Poliklinik fur Dermatologie und Allergologie, Munchen Universitaet, Munchen, Germany.

Anaphylaxis as the maximal variant of an acute systemic immediate-type allergic reaction represents one of the major emergencies in allergy. Symptoms comprise involvement of different organs (skin, respiratory tract, abdomen, cardiovascular system). Anaphylaxis may be caused through immune mechanisms or non-immunologically. Mostly, allergic anaphylaxis is mediated by IgE antibodies; however, also IgG and IgM antibodies may induce anaphylaxis through complement activation by circulating immune complexes. Non-immunological anaphylaxis (formerly also called "anaphylactoid" or "pseudo-allergic" reactions) are triggered through non-immune mechanisms mostly by drugs or additives.
Independently of the initiating event - immunological or non-immunological, mast cells and basophils are in the centre of the pathogenetic cascade leading to clinical symptoms through release of vasoactive mediators, the best known of which is histamine. Together with histamine other mediators like proteases or eicosanoids, are secreted. The mediator release from mast cells and basophils is not a cytolytic process but requires energy, calcium, and is temperature-dependent. In the periphery microcirculatory disturbance occurs with postcapillary plasma exudation, decreased capillary pressure and perfusion until stasis.
Among counter regulatory mechanisms the renin-angiotensin system seems to play a role. Patients with history of severe anaphylaxis show decreased plasma angiotensin II levels.
Treatment of anaphylaxis is mostly empiric with few evidence-based studies. A better understanding of the pathophysiological events occurring in the process after allergen has contacted IgE and the manifestation of clinical symptoms would help to introduce better therapeutic and preventive concepts.

References

Galli, S. (ed.) Anaphylaxis. Novartis Foundation Symposium 257, Wiley, Chichester (UK) 2004.
Ring J, Behrendt H. Clin Rev Allergy Immunol 17 (1999) 387-399.

Oral communications

[1] O

Dopaminergic excitation of histaminergic tube romamillary neurons

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The role of dopamine in sleep-wake regulation is unclear, despite the fact that psychostimulants cause behavioural arousal and motor activation. We investigated the interaction of dopamine with the wake-promoting histaminergic system. Immunocytochemistry revealed localization of tyrosine hydroxylase-positive cell bodies (dopaminergic neurons) and fibres in close proximity to the histaminergic neurons in the dorsolateral tuberomamillary nucleus (TMN) neighbouring the substantia nigra. Double stainings for D_2 or D_1 receptors with histidine decarboxylase failed to demonstrate these receptors on TMN neurons in slices as well as primary cultures made from the posterior hypothalamus, but many glial cells and fibroblasts were stained for D_2 and D_1 receptors. The firing rate of pharmacologically identified TMN neurons in rat hypothalamic slices was enhanced by quinpirole (100μM, D_2-receptor-agonist). This activation was abolished by sulpiride (10μM, D_2-receptor-antagonist). Participation of nitric oxide, glutamate, ATP and growth factors, which could be responsible for the neuronal excitation through D_1/D_2 receptor activation of non-neuronal elements (glia or fibroblasts) was excluded as a major mechanism. Calcium imaging with fluo4 performed in primary cultures containing TMN neurons in the presence of TTX revealed an immediate calcium rise in some neurons upon perfusion of quinpirole followed by a delayed (5 min) induction of calcium oscillations in some non-neuronal cells which decayed within 10-30 min. We conclude that quinpirole directly excites TMN neurons through D_2-like receptors.

[2] O

Respective role of histamine and orexin neurons in sleep-wake control

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The posterior hypothalamus has been recognized for its importance in maintaining waking (W). Our previous studies suggest that this role is mediated, in part, by the widespread projecting histamine(HA) neurons. The identification of orexin cells adjacent to HA neurons and their diffuse projections strengthens the idea that multiple neuron populations are involved in the hypothalamic control of the sleep-Wstates. This study was designed to determine the respective role of HA and orexin neurons in W control using histidine-decarboxylase (HDC, HA synthesizing enzyme) and orexin knockout(KO) mice.
Male adult HDC(n=9) and orexin KO mice(n=15) and their wild-type(WT) genotypes were simultaneously investigated using multiple approaches: polygraphic sleep-W recording, analysis of EEG power spectre, HDC and orexin gene PCA identification, HA and orexin immunohistochemistry, pharmacological administration and behavioral tests.
HDC-KO and orexin-KO mice share some phenotypes, such as mild obesity and an increase in paradoxical sleep(PS), but are distinct in terms of the following phenotypes: 1) The PS increase in HDC KO mice was seen during the light-period, whereas that in orexin KO mice occurred during darkness; 2) Only HDC-KO mice showed a deficit of W around lights-off, accompanied by an impaired EEG; 2) Both WT and orexin KO mice were able to respond to a new environment with increased W, whereas HDC-KO mice fell asleep and showed signs of somnolence faced with various behavioral stimuli; 3) orexin KO, but not their littermate WT or HDC KO mice, displayed signs of narcolepsy and failed to respond to a motor challenge (wheel test) with increased W and locomotion.
We hypothesized that HA and orexin neurons exert a synergistic/complementary control during W: the amine being mainly responsible for its qualitative aspects, EEG arousal and cognitive activities; whereas the neuropeptide more involved in its behavioral aspects(e.g., locomotion, food intake) and emotional reactions.

[3] O

Detection of functional heterogeneity of histaminergic neurons in response to GSK189254, a novel H_3 receptor antagonist

Patrizia Giannoni[1], Maria Beatrice Passani[1], Daniele Nosi[2], Andrew D. Medhurst[3], Paul Chazot4, Fiona Shenton[4], Patrizio Blandina[1]
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Neuronal histamine (HA) is released from axon varicosities innervating the entire brain, and originating within the only source of HA fibers, the tuberomammillary nucleus (TMN). To learn whether functional differences exist among HA neurons projecting to different brain areas, SD rats were implanted with one probe in the TMN, and one in the nucleus basalis magnocellularis (NBM), nucleus accumbens (NAc), dorsal striatum (DS) or prefrontal cortex (PFC). HA output from the two probes, perfused with Ringer at 2-µl/min, was measured in 15-min samples by HPLC-fluorometric detection. We reported that intra-TMN administration of drugs acting directly on HA neurons (thioperamide or bicuculline) affected selectively HA neurons projecting to different areas [1]. Here, we show the effects of a novel H_3 receptor (H_3R) antagonist, GSK189254. Spontaneous HA release from all regions was stable, ranging 0.05-0.08 pmol/15min (N=18). GSK189254 (1µM), infused for 60 min into the TMN, increased HA release from the TMN and PFC, but not from the DS nor NAc. These results demonstrate that H_3R blockade does not activate all HA neurons. Significant effects were determined by ANOVA/Fisher's test. To add strength to this observation, we examined H_3R distribution on HA neurons. Hypothalamic slices were labelled with anti-H_3R (349-358) [2,3]- and anti-histidine decarboxylase (HDC)-antibodies. Confocal analysis showed HDC-positive cell bodies or dendrites strongly immunopositive for H_3R, and HDC-positive cell bodies or dendrites weakly or not immunolabelled for H_3R, thus suggesting that not all HA neurons carry H_3R. H_3R ligands may be important in the treatment of cognitive deficits, sleep disorders and obesity. Recruitment of HA subpopulations may achieve more selective actions, and reduce collateral effects.

[1] Giannoni P et al, 35th EHRS Meeting, 10-13/5/2006, Delphi, GR.
[2] Chazot PL et al, Neuroreport 12, 259 (2001).
[3] Cannon KE et al, Pain (2006) Nov 27; [Epub ahead of print].

[4] O

The H_4 histamine receptor is expressed in both the human CNS and rodent PNS

Paul L. Chazot[1], Fiona C. Shenton[1], Henry Waldvogel[3], Daniela Grandi[2] and Giuseppina Morini[2].
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The histamine H_3 receptor is a presynaptic auto- and hetero-receptor, reported to be abundantly expressed in the central nervous system of different mammalian species, including human, while the presence of the histamine H_4 receptor in neurons remains controversial. This present study is aimed to investigate the expression of histamine H_4R in the human brain and rodent gastrointestinal tract, using immunohistochemical (IHC) and immunoblotting (IB) methods with our anti-H_4R (374-390) antibodies raised in rabbits, affinity purified and validated as previously described1. For the human study, a major immunoreactive (IR) protein species co-incident with the dimeric H_4R expressed in human spleen, was detected in human striatum by IB. Strong H_4R-IR punctuate decoration of putative neurons was observed in both cortical and striatal regions of human brain slices by IHC. For the rodent study, groups of rats were either fed ad libitum or fasted for 24 h before sacrifice. Tissue samples were taken from stomach, small intestine and colon, fixed in 10% formalin, and prepared for IHC. Notably, H_4R-IR was present in the myenteric of all regions of the gastrointestinal tract examined, with no apparent difference between fed and fasted animals. Staining for H_4R was particularly strong in ganglion cell soma and present, although to a lesser degree, on neuronal fibres. By contrast, the submucosal plexus was immunonegative for H_4R. This suggests a role for H_4R in the control of rhythmic peristalsis, as well as endocrine control of acid secretion and growth hormone release2. Herein, we provide the first evidence that the H_4R is expressed on neurons in both the central and autonomic nervous system, which indicates that the H_4R subserves multiple distinct roles, in addition to the control of inflammation.

[1]R Van Rijn*, PL Chazot*, FC Shenton, RA Bakker and R Leurs (2006) Mol. Pharmacol. 70, 604-615. Homo and hetero-oligomerisation of the human histamine H4 receptor (*equal contribution)
[2]G Morini, D Grandi, G Becchi, FC Shenton and PL Chazot (2007) EHRS (this meeting) Histamine H3 and H4 receptors are expressed on distinct endocrine cell types in the rat fundic mucosa.

The presenting author wishes to thank the Wellcome Trust (UK) for financial support.

[5] O

The roles of histamine receptors on cognition

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Recent studies indicate that the histaminergic system is critical for wakefulness. It is well known that sedative antihistamines induce cognitive decline through blockage of H_1 receptor in humans. In contrast, both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavioral studies. The aim of this study was to investigate the role of histamine H_1 and H_2 receptors in cognition in physiological and pathological conditions using H_1 and H_2 receptors genes knockout mice (H_1KO and H_2KO, respectively). In normal condition, several behavioral studies indicated that both H_1KO and H_2KO show impaired object recognition and spatial memory, improved conditioned fear memory. Moreover, hippocampal long-term potentiation was reduced in both H_1KO and H_2KO. These results indicate that H_1 and H_2 receptors are synergistically involved in memory process for which the frontal cortex, amygdala and hippocampus interact. In conclusion, t! he blockage of H_1 and H_2 receptors impairs cognition in normal conditions. In pathological conditions, H_1KO and H_2KO were subjected to social isolation just after weaning. After 4-week isolation behavioral and neurochemical changes were evaluated as an animal model of schizophrenia. Social isolation impaired locomotion in home-cage, prepulse inhibition of startle response and water maze performance in control mice, but not in H_1KO. Mutation of H_1-receptor decreases isolation-induced hyperactivity of cortical dopaminergic neurons. H_2KO showed similar changes to H_1KO, but the alteration in H_2KO was statistically insignificant. These findings indicate that blockage of H_1-receptor attenuates social isolation-induced cognitive impairment, suggesting that the effects of histamine receptor blockage on cognition are states-dependent.

References

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Psychopharmacology 183: 285-293 (2005).

[6] O

Assessing the potential of novel histamine H_3 receptor antagonists for the treatment of multiple CNS disorders

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H_3 receptors are widely expressed in the mammalian CNS including areas involved in cognitive processes, arousal, and pain sensation. Blockade of H_3 receptors with selective antagonists can increase the release of neurotransmitters including histamine, acetylcholine, dopamine and noradrenaline. Currently a number of H_3 antagonists are in clinical trials for various disease indications, although no efficacy data has yet been reported in patient populations.
We have assessed the effects of novel, potent and selective H_3 receptor antagonists from different chemical series in animal models of cognitive performance, sleep-wake behaviour and pain transmission. Significant improvements in the performance of rats were observed following oral administration of novel H_3 antagonists in the attentional set shift, object recognition, water maze and passive avoidance models of cognitive function, whilst transient increases in wakefulness and corresponding decreases in slow wave sleep were observed using EEG analysis. In addition, these novel H_3 antagonists significantly reversed capsaicin-induced reductions in paw withdrawal threshold, suggesting that blockade of H_3 receptors may be able to reduce tactile allodynia. These data suggest that novel H_3 antagonists may be useful for the treatment of a number of CNS disorders including dementia, narcolepsy and neuropathic pain.

[7] O

Excitatory effect of histamine on neuronal activity of rat cerebellar fastigial nucleus in vitro

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The cerebellar fastigial nucleus (FN) holds an important role in motor control and body balance. Previous studies have revealed that the nucleus is innervated by direct hypothalamocerebellar histaminergic fibers. However, the functional role of histaminergic projection in cerebellar FN has never been established. In this study, we investigated the effect of histamine on neuronal firing of cerebellar FN by using slice preparations. Sixty-five FN cells were recorded from 47 cerebellar slices, and a vast majority of the cells responded to histamine stimulation with an excitatory response (58/65, 89.2%). Perfusing slices with low-Ca^{2+}/high-Mg^{2+} medium did not block the histamine-induced excitation (n = 10), supporting a direct postsynaptic action of histamine on the cells. The excitatory effect of histamine on FN neurons was not blocked by selective histamine H_1 receptor antagonist triprolidine (n=15) or chlorpheniramine (n=10), but was effectively suppressed by ranitidine (n = 15), a selective histamine H_2 receptor antagonist. On the other hand, selective histamine H_2 receptor agonist dimaprit (n=20) instead of histamine H_1 receptor agonist 2-pyridylethylamine (n=16) mimicked the excitatory effect of histamine on FN neurons. The dimaprit-induced FN neuronal excitation was effectively antagonized by selective histamine H_2 receptor antagonist ranitidine (n=13) but not influenced by selective histamine H_1 receptor antagonist triprolidine (n=15). These results demonstrate that histamine excites cerebellar FN cells via the histamine H_2 receptor mechanism and suggest that the hypothalamocerebellar histaminergic fibers may modulate cerebellar FN-mediated sensorimotor integration through their excitatory innervations on FN neurons.

[8] O

On the role of histamine in ethanol preference

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Previous studies have shown that an alcohol-preferring rat strain (AA) displays significantly higher brain histamine levels and turnover than the alcohol-avoiding strain (ANA). Alcohol consumption of the AA rats is also bidirectionally regulated by histamine H_3 receptor ligands (Lintunen et al. FASEB J 2001). To gain further insights in the possible role of histamine in ethanol preference and fluid balance, a mouse model which lacks the histamine-synthesizing enzyme histidine decarboxylase (HDC) was tested. Oral alcohol self-administration and preference were examined using a two-bottle choice protocol. In HDC knock-out mice, ethanol, saccharin or quinine consumption did not differ significantly from control animals, although total liquid consumption was significantly higher compared to wild type mice (174.5±1.5 g/kg/day versus 161.4±1.2 g/kg/day, $p<0.001$). There were significant decreases in ethanol preference only at the lower alcohol concentrations, supporting the concept that histamine is not directly or alone involved in ethanol preference. Blood ethanol levels and thus the ability to metabolise alcohol were not different between HDC knock-out and wild type mice. The results also suggest that histamine is important in regulation of fluid balance.

Supported by the Academy of Finland and the Finnish Foundation for Alcohol Studies.
We thank Professor Hiroshi Ohtsu for the HDC knock-out mouse line.

[9] O

Molecular determinants of ligand-directed Gq versus Gs signaling for the histamine H_1 receptor

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The H_1 GPCR can activate Gq to stimulate phospholipase C (PLC) signaling or Gs to stimulate adenylyl cyclase (AC) signaling. Generally, H_1-mediated activation of PLC signaling is undesirable because it leads to bronchial and GI smooth muscle contraction, inflammation, and other negative effects of allergic responses. H_1-mediated activation of AC signaling, however, modulates brain and adrenal catecholamine neurotransmitter synthesis. Development of H_1 agonist drugs that selectively modulate H_1/AC signaling may provide for novel pharmacotherapy of neuropsychiatric and cardiovascular diseases. We have discovered ligands that selectively activate H_1/Gq/PLC vs. H_1/Gs/AC signaling and here we begin to report molecular determinants for H_1/Gq/PLC vs. Gs/AC signaling. We undertook mutagenesis studies of the human H_1-receptor, focusing on 14 specific amino acids in transmembrane domains 3-7, hypothesized to be involved in ligand binding and function. Each of the 14 point-mutated human H1 receptors was expressed in CHO cells. Ligand binding was abolished at D3.32A, Y3.33A, W4.56A, F5.47A, Y5.48A, W248A, Y6.51A, F6.55A, Y7.43A, and Y7.53A mutated H_1 receptors, but, retained at K5.39A, T5.42A, and N5.46A receptors. For K5.39A H_1 receptors, histamine H_1/PLC signaling is reduced by 50% but H_1/AC signaling is increased 200%, suggesting K5.39 contributes to H_1 conformational changes involved in Gq vs. Gs signaling. For the N5.46A H_1 receptor, histamine can displace ^{3}H-mepyramine but not the H_1 dimer radiolabel ^{3}H-PAT, suggesting N5.46 is critical for activation of H_1 dimers. Consistent with binding results, histamine maximal efficacy to stimulate PLC signaling in CHO cells expressing N5.46A H_1 receptors is only 15% WT efficacy, suggesting dimerization influences Gq signaling. These results begin to establish molecular interactions that occur between ligands and H_1 amino acids that result in H_1/Gq/PLC vs. H_1/Gs/AC signaling.

[10] O

Histamine H_3 and H_4 receptors are expressed on distinct endocrine cell types in the rat fundic mucosa

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The present study was designed to explore the expression and distribution of histamine H_3 and H_4 receptors within the rat gastric fundus by immunohistochemistry using our unique immunological probes. Groups of rats were either fed ad libitum or fasted for 24h before the sacrifice. Tissue samples from the fundic region of the stomach were removed, fixed and processed for immunohistochemistry, using our validated anti-H_3 (349-358) and anti-H_4 (374-390) antibodies (Cannon et al., 2006; Van Rijn, Chazot et al., 2006).
The results showed that cells immunoreactive for H_3R and H_4R are located in the fundic epithelium. The vast majority of H_3R immunopositive cells occupied the lower half of the glands, and were absent in the superficial portion. Their number per gland was 5.38 ± 0.19 in freely fed rats and 3.21 ± 0.40 in fasted rats. Conversely, cells immunoreactive for H_4R were dispersed throughout the glands. Their number per gland was 0.75 ± 0.03 in fed rats and 0.90 ± 0.05 in fasted rats. Double immunostaining revealed that the H_3R is not colocalized with the H_4R, while both H_3R and H_4R positive cells were immunoreactive for chromogranin A, suggesting that these immunopositive cells are endocrine cells. Because five different endocrine cell types have been identified in the rat fundic epithelium, we used double immunostaining to identify the cell types expressing H_3R and H_4R, respectively. Approximately 90% of cells positive for H_3R were also immunoreactive for histidine decarboxylase, demonstrating that the H_3R is located on ECL cells. Cells positive for H_4R were immunoreactive for ghrelin, originating from A-like cells.
These findings may imply that histamine, via a balanced interaction with H_3R and H_4R expressed on ECL and A-like cells respectively, regulates not only acid secretion and mucosal protection but could also have a role in feeding behaviour and growth hormone release.

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R Van Rijn*, PL Chazot*, FC Shenton, RA Bakker and R Leurs (2006) Mol. Pharmacol. 70, 604-615. Homo and hetero-oligomerisation of the human histamine H4 receptor (* equal contribution).

[11] O

Histamine H_4 receptor species variants and the molecular basis for their pharmacological differences

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The human histamine H_4 receptor (H_4R) shows moderate to high sequence homology to the H_4R proteins of several animal species (mouse, rat, guinea pig, pig, dog, and monkey). The differences in protein structure lead to substantial differences in pharmacological profiles for tested H_4R ligands, like the H_4R agonists histamine, clozapine, and VUF 8430 and H_4R antagonist JNJ 7777120. The H_4R ligands show differences in H_4R affinity, but also in their functional activities at the species variants of the H_4R.
We initially constructed chimeric receptors of the human and mouse H_4R to address the molecular basis for the different affinity of histamine for these two receptors. This effort resulted in the finding that the second extracellular loop of the H_4R is responsible for the observed difference. We continued these investigations with the analysis of single point mutants, which allowed us to pinpoint the residue(s) that play important roles in the pharmacological differences.
Our mutagenesis effort was also broadened in order to address the pharmacological differences between the human H_4R and the other species variants. As a result, we were able to pinpoint amino acid residues that cause pharmacological differences at the various species variants for histamine, clozapine, VUF 8430, and JNJ 7777120. These results are all highly valuable for the construction of a validated model of the histamine H_4R.

[12] O

MS analysis of human histamine H_1 receptors

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During the past decades, MS analysis is standing out as the method of choices to determine protein-protein interaction and ligand-protein interactions. However, due to the low abundance and the hydrophobic nature of GPCRs, limited information is available on ligand-GPCR interactions. Here we reported the first successful use of MS analysis to directly reveal the primary structure of the human H_1R protein, obtained using both cell free and cellular expression systems. Using our optimized digestion procedure, more than 80% of protein sequence, including five TM domains, was identified in the MS analysis. This approach also allows us to further study the protein-ligand interaction, by covalently labeling of the H_1R protein with irreversible histaminergic compounds. In combination with data obtained through homology modeling and site directed mutagenesis, these approaches will enhance our knowledge regarding the tertiary structure of this archetypal GPCR and its interaction with small molecules.

[13] O

Effects of MAPK inhibitors on histamine and eicosanoid generation from human lung mast cells

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The potential involvement of the mitogen-activated protein kinases (MAPK) p38, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in the IgE-dependent release of mediators from mast cells was investigated. To this end, the effects of selective inhibitors of p38 (SB202190), ERK (PD98059) and JNK (SP600125) on the IgE-mediated generation of histamine, cysteinyl-leukotrienes (cys-LT) and prostaglandin D2 (PGD_2) from human lung mast cells were determined. Mast cells were isolated from physically and enzymatically disrupted human lung tissue. Cells were incubated with or without a MAPK inhibitor (0.1-30 μM) for 1 h before challenge with anti-IgE (1:300) for 25 min. Histamine released into supernatants was determined by an automated fluorometric technique. The production of cys-LT and PGD_2 was determined using commercially-available ELISA kits. Maximal inhibitory responses and potencies of the inhibitors were determined by non-linear regression analysis (GraphPad Prism, version 4).
Values cited are means±SEM, n = 4-10.
Although SP600125, SB202190 and PD98059 were roughly equipotent (logEC50 ~ -5.6) as inhibitors of IgE-dependent histamine release from mast cells there was a clear difference in the efficacy of the compounds since the maximal % inhibition was 76±7, 41±6 and 23±5%, respectively. The MAPK inhibitors, at higher concentrations, completely abolished the IgE-mediated generation of cys-LT although SP600125 (-6.1 ±0.4) was slightlyork more potent than SB202190 (-5.8±0.2) and 10-fold more potent than PD98059 (-5.2 ±0.1). The MAPK inhibitors also completely blocked IgE-dependent PGD_2 generation and SP600125 (-5.3±0.1), SB202190 (-5.5±0.1) and PD98059 (-5.6±0.3) were roughly equipotent.
These findings with selective MAPK inhibitors suggest that p38, ERK and JNK may all influence the generation of mediators from mast cells. However, the relative contribution of each of these MAPK to the elaboration of a given mediator may vary.

[14] O

Cimetidine induces IL-18 production through H_2-agonist activity in monocytes

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The present study demonstrated possible mechanism for the improvement of gastrointestinal cancer patients' prognosis by a histamine receptor type2 (H_2R) antagonist, cimetidine. This agent, but not the H_2R antagonists, ranitidine and famotidine, induced the production of anti-tumor cytokine, interleukin (IL)-18 by human monocytes and dendritic cells (DC). In fact, cimetidine-induced IL-18 production was antagonized by ranitidine and famotidine. Cimetidine induced the activation of caspase-1, which is reported to modify immature-IL-18 to mature/active-IL-18, and the elevation of intracellular cyclic adenosine monophosphate (cAMP), leading to the activation of protein kinase A (PKA). A PKA inhibitior, H89 abolished the IL-18 production induced by cimetidine. Moreover, the effects of cimetidine on IL-18 production were reproduced in peripheral blood mononuclear cells (PBMC) from wild mice, but not in those from H_2R knockout mice. In conclusion, cimetidine, a partial agonist for H_2R, has a pharmacological profile different from ranitidine and famotidine, possibly contributing to its anti-tumor activity on gastrointestinal cancers.

[15] O

Histamine and histamine receptor antagonists as an IgE-regulatory factors

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Histamine play a crucial role in the pathogenesis of both IgE-mediated allergic as well as non-allergic diseases. The role of histamine in IgE immunoregulation is less known. Previous investigations have shown that histamine mediates IgE regulation by involvement of H_1, H_2 and H_3/H_4 receptors. In the present study the influence of histamine and several histamine receptor antagonists was studied in mononuclear cell's (MNC) culture of allergic patients and patients with lympho- and erythroproliferative diseases . MNC were cultured with histamine and H_1 (loratadine), H_2 (cimetidine) and several imidazole and non-imidazole H_3/H_4 antagonists (FUB 181, FUB 649, FUB 372, Imoproxifan-IMP and Ciproxifan-CF) in concentrations 10^{-5}M and 10^{-8}M. Determination of total IgE in the supernatants performed by CAP FEIA method (Phadia). It has been shown that histamine regulates IgE production in allergic diseases as well as in non-allergic diseases such as malignant bone marrow disorders. The histamine-mediated effects on IgE synthesis are highly dependent on the concentration of mediator and type of histamine receptors involved. It have been shown that high concentrations (10^{-5}M) of histamine suppresses and low concentrations (10^{-8}M) stimulates spontaneous IgE synthesis. Antagonists of H_1 and H_2 receptors oppositely effect on IgE production in the cell culture of allergic patients and patients with lympho- and erythroproliferative diseases. IgE synthesis is highly dependent on the potency and structural differences of the H_3/H_4 antagonists. IgE synthesis induced by the H_3/H_4 antagonists depends on the preexisting serum levels as well as the synthesis of IgE. It has been shown that in asthmatic patients, having low levels of IgE synthesis, FUB 181 increased an IgE synthesis of 1.35 fold. At the same time, here was no effect on IgE synthesis in the group with the preexisting high levels of IgE ($p<0.01$). IgE modulation induced by H_3/H_4 antagonists was dependent on the cytokine synthesis. H_3/H_4 antagonist increased an IL-4 synthesis in atopic patients more than 2-fold. At the same time H_3/H_4 antagonist decreased an INF-γ synthesis and had no effect on the IL-13 production.

[16] O

Ex vivo-assessment of allergens includes the histamine liberation test (HLT)

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The current EU-REACH policy indicates carcinogenic, mutagenic, teratogenic and bio accumulative substances. The development and the use of new - but also many of the old -chemicals are responsible for further sustain increase of allergic diseases [1]. Furthermore, in many cases even traces of allergens can cause allergic diseases of the skin and/or respiratory tract. There are no available threshold values of allergens to date. The AVE has published an allergen list, which shall be used as a basic recommendation for sensitizing risk assessment in the REACH product registration [2]. The examination of chemicals and products - related to its allergenic/sensitizing potency - is demanded. Immunological tests were performed for the allegro-toxicological assessments using blood cell fractions of atopic and non-atopic human blood cell cultures (PBMC) [3]. In a retrospective study 126 matched controls (63 pairs) were examined using:
1 the Histamine Liberation Test HLT (which mimicks the anaphylactic and "anaphylactoid" immediate reaction);
2 the Basophil Degranulation Test BDT (panoptical determination of the degranulated basophils from atopic and non-atopic individuals. This is to recognize the challenge of basophils in atopic and non-atopic samples);
3 the Lymphocyte Stimulation Test LST (testing the lymphocyte proliferation and cytokine production, after stimulation using different mitogens like PHA - phytohemagglutinin or anti-CD3). In 70 % the HLT revealed significantly increased histamine liberation ($p < 0.05$) in the atopic samples. The BDT showed 68 % and the LST 81 % elevated responses, respectively. It can be suggested that the HLT, combined with the BDT and the LST are a good approach and practical human ex vivo/in vitro methods to estimate the allergenic/sensitizing potency of products.

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[3] Diel et al. (1998) Allergy 53, 1052-1059.

[17] O

Histamine receptor H_4R-selective ligands influence the STAT6 Transcription Activation Domaine (TAD) and the DNA-binding

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In recent work the JAK/STAT downstream regulation was investigated in sensitized human lymphocytes. It could be shown that the selective H_4R antagonist JNJ7777120 abolishes the inhibitory effects of histamine on the STAT1 downstream phosphorylation as well as on the specific interaction of STAT1 to its promotor DNA ex vivo [1]. In this study the STAT6 DNA interaction was examined using JNJ7777120 and the benzoimidazol derivative JNJ10191584 [2]. Sensitized human PBMC served as an ex vivo model as described before [1]. Histamine, JNJ7777120 and JNJ10191584 (gift from RJ Thurmond, Johnson&Johnson, Pharmaceutical Research and Development, L.L.C.) were added (1 and 10 μM) alone or in combination. DNA-binding was measured by the electrophoretic mobility shift assay (EMSA) using STAT1 oligonucleotide (5´-CATGTTATGCATATTCCTGTAAGTGAAAA-3´; Metabion) and STAT6 oligonucleotide (5´-TAGTCAACTTCCCAAGAA CAGAATCA-3´) respectively. Histamine significantly inhibited the STAT1 DNA as well as the STAT6 DNA-binding (35 - 50%) in sensitized, un-sensitized PBMC ($p < 0.05$). Experiments with Jurkat cell line (gift from KH Friedrich, Jena) resulted in similar data. Both neutral H_4R antagonists, JNJ7777120 and JNJ10191584, abolished the responses of histamine and the interaction of STAT6 oligoDNA was enhanced even more effectively using JNJ10191584 (10 μM). In good agreement with our recent data, the interaction of STAT6 oligoDNA was increased in sensitized cells. This was also true measuring the transcription activation domain (TAD) of STAT6 after 3 days incubation time. Western blot technique provided for the identification of the TAD after nuclear enzymatic cleavage. In the presence of 1 μM histamine TAD STAT6 oligoDNA binding was increased in a comparable extend as the STAT6 oligoDNA electrophoretic band (EMSA) disappeared. It can be suggested that H_4R - as a member of the G-protein coupled receptor (GPCR) super family - influences the TAD cleavage and that histamine can down-regulate the transcription activity of STAT6. However, the STAT1/STAT6 balance must also be taken in consideration. It can be adjusted by H_4R-selective anti-histamines.

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[2] Dunford et al. J Immunol 2006;176:7062-7070.

[18] O

Effects of c-kit receptor mutation on the maturation and the function of rat peritoneal mast cells: using heterozygous Ws/+ rats

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The interaction between the c-kit receptor and its ligand, stem cell factor (SCF) results in Kit signaling via phosphorylation of a tyrosine residue in the c-kit receptor and plays important roles in the proliferation and differentiation of mast cells. We compared heterozygous rats (Ws/+) in which one Ws (white spotting) allele is mutated with congenic normal rats (+/+) to determine the effect of the c-kit receptor on the proliferation of and functional changes in mast cells. Peritoneal mast cells (PMCs) of Ws/+ and +/+ rats originated from Donryu rat strain (250-300g) were purified according to the density gradient methods using 38 % bovine serum albumin (BSA). To check the expression of mutated c-kit receptor gene (Ws) which is characterized by the deletion of 12-nucleotides in the tyrosine kinase domain, P-CR products of c-kit receptor were analyzed using GAPDH as a control. After co-cultured with NIH 3T3 fibroblasts, PMCs were challenged immunologically or non-immunologically, with dintrophenylated-BSA (DNP-BSA) as an antigen and compound 48/80, respectively. Histamine contents of PMCs and histamine release from PMCs were determined by HPLC-fluorometry. A single band of 218 bp was observed in +/+ PMCs, whereas, two distinct bands of PCR products were detected in Ws/+ PMCs. The number of PMCs and the histamine content of PMCs of Ws/+ rats were 2.35 x105 /rat and 0.181 pmol/cell, respectively, which was 37.2 % and 89.1 % of the values in +/+ rats. The histamine release after stimulation with the antigen and compound 48/80 showed no significant difference between the two groups of rats. The comparison of PMCs derived from Ws/+ and +/+ rats showed that the decreased Kit signaling due to the mutation of c-kit receptor caused changes in proliferation and maturation, but not in the exocytotic function. Furthermore, the co-culture of PMCs with NIH 3T3 fibroblasts proved to be a useful method for studying CTMCs morphologically and functionally.

[19] O

Beneficial effects of a plant histaminase in a rat model of splanchnic artery occlusion and reperfusion

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Splanchnic artery occlusion (SAO) followed by reperfusion causes endothelial injury and inflammation which contribute to the pathophysiology of shock. Histamine secretion from storage and producing cells, mainly intestinal mast cells, in the extracellular compartment and in blood contributes to lethal circulatory shock occurring upon SAO. Histamine is released by the intestinal mast cells mostly during reperfusion and sparkles a vicious cycle that leads to further endothelial activation and leukocyte adhesion and extravasation. We investigated the effects of pea seedling (Latyrus cicera) histaminase, known to afford protection against the deleterious effects of cardiac ischemia/reperfusion [1], 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.

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[20] O

Characterization of human plasma amine oxidase

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Human blood plasma contains a soluble copper-containing amine oxidase (AOC) whose activity is rather low as compared with other mammalian species. Recently we could show that in most mammals the major fraction of plasma amine oxidase is the product of the AOC4 gene, which encodes a soluble, secretory protein mainly expressed in the liver. Humans and rodents lack a functional AOC4 gene and their low constitutive plasma amine oxidase activity presumably results from partial proteolytic release of the large extracellular portion of the membrane-associated AOC3 gene product called vascular adhesion protein-1 (VAP-1). In order to learn more about the function of human plasma amine oxidase we purified the protein and determined its molecular and enzymatic properties. Human plasma amine oxidase is a homodimeric glycoprotein of ca. 200 kDa. In contrast to the porcine plasma enzyme that converts monoamines, diamines, polyamines, histamine, and N-methylhistamine, the human enzyme oxidizes various mono- and diamines but not histamine. The low activity of plasma amine oxidase in humans and its inability to convert histamine are associated with a higher sensitivity for plasma histamine in man compared to other mammals. In humans, the AOC1 gene product diamine oxidase (DAO) is solely responsible for the inactivation of extracellular and specifically plasma histamine by oxidative deamination.

[21] O

Possible pharmacological applications of copper amine oxidases

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Copper amine oxidases (Cu-AOs) are animal or vegetal enzymes able to oxidize a wide variety of amines (i.e. histamine, polyamines) to hydrogen peroxide and aldehydes. As either the substrates and the products of their catalytic reaction are important modulators of cellular functions, these enzymes might have possible pharmacological applications. AO might be used to control cellular proliferation. Intraperitoneal tumour growth was reduced after injection of ConA-Sepharose-immobilized diamine oxidase (DAO), purified from pig kidney, into the peritoneal cavity in Swiss mice (Mondovì B et al. Agents Actions 12:450-451, 1982) and cytotoxicity of bovine serum AO, added to culture medium in the presence of spermine, was observed in Chinese hamster ovary cells (Averill-Bates D et al. Biochem Cell Biol 72:36-42, 1994). Furthermore, Cu-AOs might represent new therapeutic strategies for allergic asthma and inflammation. Free or CNBr-Sepharose-immobilized Cu-AO from pea seedlings, given intraperitoneally or by aerosol, significantly reduced the severity of cough, the occurrence of dyspnea and delayed the onset of respiratory abnormalities in sensitized male albino guinea pigs (Masini E et al. Eur J Pharmacol 502:253-264, 2004). Also, in a rat model of ulcerative colitis intraperitoneally administrated DAO reduced the inflammation and accelerated the healing of damaged mucosa (Fogel WA et al. Inflamm Res 55 Suppl 1:S68-69, 2006). Cu-AO might also be used as a cardioprotective agent. Vegetal enzyme exerted protective effects in a rat model of myocardial ischemia and reperfusion injury (Masini E et al. Biochem Biophys Res Commun 309:432-439, 2003) and modulated the cardiac anaphylactic response in isolated hearts from guinea pig (Masini E et al. Biochem Biophys Res Commun 296:840-846, 2002). Beneficial effects on splanchnic artery occlusion and reperfusion were also obtained in a rat model (Cuzzocrea S. et al., this meeting).

[22] O

Methylamine, an endogenous regulator of adipocyte nitric oxide synthase activity

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Background: Adipocytes from different animal species express high levels of a semicarbazide-sensitive amine oxidase activity (Bz-SSAO) endowed of histaminase activity whose physiopathological meaning has been not clarified yet. Among the different Bz-SSAO substrates is methylamine (MET), the smallest ammonia alkyl derivative endogenously occurring from diet and from protein degradation whose levels increased in several physio-pathological conditions including diabetes and neurodegenerative diseases (Yu, 1998; del Mar et al., 2005). We described recently that MET is endowed of hypophagic effects in rodents increasing hypothalamic levels of nitric oxide (NO) and dopamine. Aim: Since adipocytes contain both the endothelial and the inducible isoform of the nitric oxide synthase, differently involved in insulin resistance (Engeli et al., 2004), we aimed to verify whether MET was able to induce also in this peripheral tissue-derived cells, NO production. Results: MET (from 0.5 to 5 mM) increases the basal (1.4 ± 0.5 pmol/10^4cells) NO production from isolated adipocytes with an half maximum effective concentration (EC50) of 1.9 ± 0.2 mM. MET-dependent NO production is followed by increase of cGMP levels and it was inhibited by L-NAME (10-4) indicating the activation of the NO synthase. MET-induced NO production was dependent on extracellular [Ca^{2+}]. Inhibiting the Bz-SSAO activity, MET-dependent NO production increased. Conclusions: MET directly activates the endothelial nitric oxidase synthase activity of adipocytes. Notwithstanding the impact of such function on adipocyte physiology remains to be investigated, Bz-SSAO inhibitor might be proposed as pharmacological tools to increase adipocyte NO levels.

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[23] O

Selective H_3 receptor isoforms activate Akt and ERK in rat cortical neurones

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The H_3 receptor (H_3R) shows functional constitutive activity, polymorphisms in humans and rodents, differential distribution of splice variants in the CNS and potential coupling to different intracellular signal transduction mechanisms.
We previously showed that H_3R agonists induce a time dependent activation of the Akt/GSK axis in rat embryonic day (ED) 17 cortical neurones cultured for 6-8 days. This effect peaks at 60 min (Mariottini et al., 2006 XXXV EHRS Meeting, Delphi GR). We now demonstrate that incubation of cortical neurons with the H_3R agonist immepip (10 nM) induces phosphorylation of ERK 1/2 that reaches a maximum at 15 min. ERK activation was inhibited by the MEK inhibitor UO126 (10 μM) and the H_3R antagonist/inverse agonist thioperamide. Also, UO126 decreased Akt/GSK phosphorylation after 60 min of incubation with immepip, suggesting an interaction between the Akt- and ERK- mediated transduction cascades. Constitutive H_3R activation of Akt was not observed in cultured neurons, when incubated with 10 μM thioperamide. As Gi-coupled receptors were shown to increase intracellular Ca^{2+} concentration ([Ca++]i), cortical neurons loaded with Fluo-4 were stimulated with 1 μM immepip. Calcium imaging analysis showed no detectable changes in [Ca^{2+}]i. Using RT-PCR we found that cortical neurons express two out of the three functional H3R isoforms, namely A and C. Consistent with previously published data (Drutel et al. Mol Pharmacol 2001 59:1-8), the primers used recognised isoforms A, B and C in adult brains.
H_3R ligands affect cognition, wakefulness, obesity and epilepsy, which are physiological and pathological conditions that are the main focus of research into the therapeutic potential of selective H_3R ligands. Given the molecular and pharmacological heterogeneity of the H_3R, it is important to understand if and which transduction cascades are associated with different H_3R isoforms in light of more selective therapeutic treatments devoid of unwanted side effects.

[24] O

Increased antigen presentation and Th1-polarization in genetically histamine-free mice

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Dendritic cells (DCs) are the main antigen presenting cells. Their main function is antigen processing and presentation, which can be affected by several environmental factors such as histamine. Using a genetically histamine-free (histidine-decarboxylase knock out, HDC-/-) mouse model, we examined the effects of histamine on DC-mediated antigen presentation and cytokine production. We found that spleen DCs, derived from HDC-/- mice, display a higher efficiency in antigen presentation compared to wild type cells. After flow cytometric analysis of the main DC cell surface markers and costimulatory molecules we found that these two DC groups do not differ in their phenotype or maturation status. We also analyzed their cytokine expression profile by real time PCR. After in vivo CFA treatment we measured enhanced Th1 cytokine profile (IL-12p35 and IFNγ) in HDC-/- DCs compared to the wild type ones. In vitro investigations confirmed that isolated DCs, developed in the absence of h! istamine, exhibit indeed a predominantly Th1 polarized cytokine pattern, as they show elevated levels of IFNγ mRNA upon LPS stimulation. Similar difference was found at the protein level by ELISA, as well. Our study demonstrates that histamine interferes with antigen presentation and alters the cytokine profile of dendritic cells.

[25] O

Differential regulation of IgE-mediated histamine release in human basophils by SHIP-1 and syk

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IgE-mediated signalling in basophils is thought to involve the early activation of the tyrosine kinase Syk, and decreased Syk expression has been reported to be responsible for a non-histamine-releasing basophil phenotype. In this study we compared Syk activities and expressions to those of the inhibitory phosphatase, SHIP-1, in order to discover whether SHIP-1 also contributes to the lack of histamine release observed in non-responder basophils. Human basophils were highly purified (92-100 % purity) by Ficoll-density centrifugation, elutriation and negative selection using magnetic cell sorting. Basophils from a range of donors were treated with and without anti-IgE, followed by assessment of histamine releases using spectrofluorometric analysis and Western blotting to determine signal enzyme expressions and activities. Syk phosphorylation in non-responder basophils was not increased after anti-IgE stimulation in contrast to basophils which released more than 15 % of their histamine content. However, phosphorylation of SHIP-1 was observed in anti-IgE-stimulated non-responders, and was generally greater than that of responders. Interestingly, basal SHIP-1 activities were significantly higher in non-responders than responders, of which the latter displayed lower SHIP-1 protein expressions (as a percentage of β-actin). These studies show that basophil reactivity, in terms of the ability to release histamine, depends not only upon stimulatory signal input (e.g. Syk) but also upon the reduced expression and phosphorylation of SHIP-1. Modulating SHIP-1 may therefore be considered as a desirable target for anti-allergic therapy.

[26] O

Mast cell inhibition and reduced histamine-related ventricular arrhythmias in a swine model of acute myocardial infarction upon therapeutic administration of the cardiotropic hormone relaxin

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Resident cardiac mast cells, located mainly around coronary vessels and concentrated in the right atrium close to the sinoatrial node, are the main repository of cardiac histamine. Inflammatory activation of cardiac mast cells, as occurs upon acute myocardial infarction, causes the release of histamine and prostanoids. These substances lead to severe tachyarrhythmias, cardiodepressive effects and coronary spasm, thus contributing to myocardial damage and early, lethal outcome.
The peptide relaxin, known to inhibit mast cell activation by a NO-dependent pathway, has been recently validated as a cardiotropic hormone, being produced by the heart and acting on specific heart receptors. In this study, we report on a swine model of heart ischemia/reperfusion, currently used to test cardiotropic drugs due to its similarities with human myocardial infarction, in which human recombinant relaxin (2.5 and 5 μg/kg b.wt.), given at reperfusion upon a 30-min ischemia, markedly reduced the main serum markers of myocardial damage (myoglobin, CK-MB and troponin T) and the metabolic and histopathological parameters of myocardial inflammation and cardiomyocyte injury, resulting in an overall improvement of ventricular performance (increased cardiac index) as compared with the controls. Relaxin-induced cardioprotection was associated with a reduced occurrence of severe ventricular arrhythmias and a clear-cut, significant reduction of plasma histamine (by ELISA) and cardiac mast cell degranulation (by computer-aided densitometry on Astra blue-stained histological slides). In conclusion, this study further supports the relevance of histamine in the pathophysiology of ischemia-reperfusion-induced cardiac injury and dysfunction. It also offers additional evidence for the cardioprotective effects of relaxin, which also involve mast cell inhibition, and provide background to future clinical trials with human relaxin as an adjunctive therapy to catheter-based coronary angioplasty in patients with acute myocardial infarction.

[27] O

Pathophysiological modulation of histamine release: a correlation between *in vitro* and *in vivo* data

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Isolated mast cells from different sources (rodents or humans, various anatomical sites) have been widely used for a long time to characterise an array of histamine segretagogues and modulators of the secretion. Many of the cell models are aimed to mimic the interactions that may involve mast cells because of their closeness to blood vessels, epithelia, nerves, smooth muscle cells and mucus-producing glands. The ability of parasympathetic stimulation to release histamine from guinea-pig auricle and rat ileum as well as the demonstration of a free-radical driven release of histamine are original data which suggest modulatory mechanisms of mast cell functioning that may play a meaningful role during the inflammatory process.
Neutrophil-evoked histamine release appears to be a free-radical dependent process and can be modulated by a carbon monoxide (CO) releasing molecule. This and other results support the involvement of an endogenously generated gaseous compound, CO, in the modulation of histamine release, implement the experimental evidence previously achieved with NO-donors and allow us to conclude that both NO and CO contribute to control histamine release.
The data from isolated mast cells can be transferred to experimental models of i*n vivo* pathophysiological events, such as models of cardiac ischemia/reperfusion and cardiac anaphylaxis, thus indicating that the peripheral functional networks predicted for histamine by testing isolated cells can effectively operate in vivo.

[28] O

Ceramide-driven oxidative/nitrative stress: a key pathway in asthma

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Although the mechanisms involved in the pathogenesis of asthma remain unclear, a role for inflammation, oxidative/nitrative stress and epithelial cell apoptosis has been documented. These observations, taken together with our current understanding of the ceramide metabolic pathway, have lead to our hypothesis that increased formation of the biologically active lipid, ceramide, contributes to the development of airway hyperreactivity in part through the induction of oxidative/nitrative stress. Using a well characterized *in vivo* model of allergic bronchospasm in ovoalbumin (OA) actively sensitized guinea-pigs [1,2] we found that aerosol administration of OA increased ceramide levels in the airway epithelium. The increase in ceramide levels was associated with inflammation, mast cell degranulation, deactivation of MnSOD, nitrotyrosine formation, PARP activation and epithelial cell apoptosis. Finally, ceramide *up-regulation* was associated with several histopathological abnormalities and bronchocostriction in response to OA challenge. Inhibition of *de novo* ceramide synthesis with fumonisin B1 (FB1), a competitive and reversible inhibitor of ceramide synthase and thus of *de novo* ceramide synthesis, attenuated oxidative/nitrative stress, mast cell degranulation, lung cell apoptosis and the deactivation of MnSOD. FB1 also reduced the inflammatory response and the associated respiratory and histopathological abnormalities. Taken together, our results suggest that the activation of ceramide pathway contributes to the development of airway hyperreactivity through the induction of oxidative/nitrative stress. Therefore, strategies aimed at reducing the levels of ceramide should yield promising novel anti-inflammatory and anti-asthmatic agents and as such this pathway needs to be explored comprehensively.

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[29] O

Hematopoietic proliferation and IL-3 signaling are impaired in histidine decarboxylase knock out (HDC-ko) mice

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Earlier a difference was detected in the percentages of immunophenotypically characterised bone marrow (BM) cell populations between HDC-ko and wild type (WT) mice. Also, irradiation-induced BM regeneration was delayed in histamine-free mice. In the following study the proliferative capacity of hematopoietic progenitors, as well as IL-3 signaling were compared in WT and HDC-ko mice. For BM regeneration studies, HDC-KO and WT mice were subjected to whole body gamma-irradiation of 4 Gy; BM samples were obtained in the 6th hour and on the 1st, 3rd and 7th days. The mRNA and surface expression of IL-3R in regenerating BM cell populations was determined by realtime PCR and FACS, respectively. HDC and histamine content of CD34+, Sca1+, Ckit+, STR, LTR cell populations were determined in WT mice using flow cytometry. The proliferative capacity of hematopoietic cells of HDC-ko and WT mice was measured by colony forming unit (CFU) assay and MTT assay (after IL-3-stimulation), and cell cycle analysis was performed by FACS. In both IL-3-stimulated cell cultures and regenerating BM samples, mRNA of STAT5 and MAPK was determined by realtime PCR. IL-3 signaling was studied by FACS using anti-phospho-STAT5 antibody. HDC and histamine content of all BM populations increased during regeneration, though CD34+ cells contained the largest amounts of them, while LTR cells contained the least. The CFU assay showed that the number and the size of colonies were markedly reduced in samples of HDC-ko mice, indicating slower proliferation. This finding was verified by the MTT assay and cell cycle analysis. IL-3 signaling was also impaired in histamine-free mice: the expression and activation of STAT5 as well as the increase in IL-3R expression in regenerating cells was reduced in HDC-ko mice. The increased HDC and histamine content of CD34+ cells and the reduced numbers of hematopoietic progenitors of HDC-ko mice and their impaired proliferative capacity support the delay in BM regeneration observed after irradiation. The decreased IL-3 signaling is in parallel with these results, and this could give explanation on how histamine plays a role in hematopoiesis, that is, through the regulation of cytokine receptor mediated signaling.

[30] O

Histamine H_4 receptor isoform hetero-oligomerisation and cell surface expression in vitro

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The H_4R is found predominantly on a wide range of immune cells including mast cells, monocytes and eosinophils. However, we have the first evidence that the H_4R is also expressed on neuroendocrine cells, and in selective structures within the rodent and human central and autonomic nervous system (Morini et al and Chazot et al., this meeting). The H_4R is a G-protein coupled receptor (GPCR), which undergoes alternative splicing to yield shorter isoforms, which display distinct distribution patterns. The functional role of splicing is of great interest. Furthermore, current evidence suggests that GPCRs form oligomeric structures in vivo and that this is probably important for their function. In previous work, we have demonstrated the existence of rat and human H_3 and human H_4 homo-dimers with a range of biophysical and immunobiochemical techniques, using our novel anti-H_3 and anti-H_4 antibodies [1,2]. We also reported the first evidence that the rH_3 isoforms act as activity-dependent dominant negative subunits in the brain[1]. Herein, the full length $H_4(390)$ was expressed alone or co-expressed with either of the two newly identified human isoforms, $hH_4(302)$ or $hH_4(67)$ in HEK 293 cells. Biophysical assays (FRET), pharmacological (Leurs, this meeting) and immunoprecitation experiments clearly showed that hH_4 homo- and hetero-dimers are readily formed in mammalian cells. The $H_4(302)$ and (67) isoforms alone did not bind [^{3}H]histamine or transduce a signal, and did not significantly affect the affinity of [^{3}H] histamine binding to the $H_4(390)$, however both isoforms reduced the level of binding by 55% and 30%, respectively. Quantitative surface biotinylation experiments showed that all three isoforms can reach the cell surface when expressed alone, and concur with the effects of the isoforms upon $H_4(390)$ surface receptor number.
In conclusion, we have demonstrated the ability of human H_4 splice isoforms to hetero-oligomerise, and showed that the isoforms, although they have a modest negative effect, do not prevent the cell surface expression of the full length hH_4 receptor.

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[31] O

The brain H_3-receptor as a novel therapeutic target for vigilance and sleep-wake disorders

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Brain histamine(HA)ergic neurons play a prominent role in arousal and maintenance of wakefulness (W). H_3-receptors control the activity of HA neurons through presynaptic autoinhibition. The role of H_3-receptor antagonists/inverse agonists (H_3R-antagonists) in the potential therapy of vigilance deficiency and sleep-wake disorders were studied by assessing their effects on the mouse cortical EEG and sleep-wake cycle in comparison to modafinil and classical psychostimulants. The H_3R-antagonists, thioperamide and ciproxifan increased W and cortical EEG fast rhythms and, like modafinil, but unlike amphetamine and caffeine, their waking effects were not accompanied by sleep rebound. Conversely, imetit (H3R-agonist) enhanced slow wave sleep and dose dependently attenuated ciproxifan-induced W, indicating that the effects of both ligands involve H_3-receptor mechanisms. Further studies using knockout (KO)-mice confirmed the essential role of H_3-receptors and HA-mediated transmission in the wake properties of H_3R-antagonists. Thus ciproxifan produced no increase in W in either histidine-decarboxylase (HDC, HA-synthesizing enzyme) or H_1- or H_3-receptor KO-mice whereas its waking effects persisted in H_2-receptor KO-mice. These data validate our hypothesis that H_3R-antagonists, through disinhibition of H_3-autoreceptors, enhancing synaptic HA that in turn activates postsynaptic H_1-receptors promoting W. Interestingly amphetamine and modafinil, despite their potent arousal effects, appear unlikely to depend on HArgic mechanism as their effects still occurred in HDC KO-mice. The present study thus distinguishes two classes of wake-improving agents: the first acting through non-HArgic mechanisms and the second acting via HA and supports brain H_3-receptors as potentially novel therapeutic targets for vigilance and sleep-wake disorders.

[32] O

Histamine downregulates CCL2 production by monocytes and monocyte-derived inflammatory dendritic epidermal cells (IDEC) via the histamine H_4 receptor (H_4R)

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Histamine is one of the most important mediators during atopic diseases. It is released mainly by mast cells and basophils and mediates its effects via four known receptors, H_1R-H_4R. The recently discovered H_4R is expressed by leukocytes and is thought to have an immunomodulatory function. H_4R functions include chemotaxis of several cell types and regulation of inflammatory mediator expression. We investigated the effect of H_4R-agonist binding on mediator release by monocytes and inflammatory dendritic epidermal cells (IDEC). We show here that monocytes and in vitro generated monocyte-derived IDEC express a functional H_4R. H_4R protein is significantly upregulated by interferon-g. H_4R agonists clobenpropit and 4-methylhistamine induce a Ca^{2+} mobilization in monocytes which was blocked by the specific H_4R antagonist JNJ7777120. Furthermore, histamine, clobenpropit and 4-methylhistamine effectively down-regulate CCL2 production by monocytes and IDEC which was also blocked by JNJ7777120. The downregulation of CCL2 was regulated at different levels. First, after 4 hours of incubation with H_4R agonists, CCL2 mRNA was significantly decreased. Second, intracellular CCL2 protein was elevated after 4 hours of incubation with 4-methylhistamine, indicating an inhibition of CCL2 secretion. In contrast, no evidence was found for an induction of matrix metalloproteinases that can degrade CCL2. Culture medium from H_4R agonist-stimulated monocytes induced less monocyte chemotaxis than culture medium of non-stimulated monocytes. H_4R agonists did not stimulate chemotaxis directly. In conclusion, human monocytes express H_4R protein and ligation of the receptor with specific agonists leads to a Ca^{2+} mobilization. Furthermore, H_4R inhibit CCL2 production resulting in a reduction of monocyte recruitment. Thus, the H_4R could represent an important anti-inflammatory receptor on monocytes and IDEC.

[33] O

Synthesis and SAR of heterocyclic piperazines as potent histamine H_4 receptor ligands

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Histamine, which is known to play a role in most inflammatory conditions including allergy, asthma and autoimmune diseases, exerts its actions through four known receptors. The most recently cloned histamine receptor, H_4R, has been shown to have a role in chemotaxis and mediator release in various types of immune cells including mast cells, eosinophils, dendritic cells, and T cells, suggesting that it may play an important role in inflammatory responses. The discovery of selective ligands for this receptor has been crucial in uncovering its function. To this end, several series of novel histamine H_4 receptor ligands have been synthesized and their structure-activityrelationships evaluated for activity at the H_4 receptor. Three of these series are derived from indoly-2-yl-(4-methyl-piperazin-1-yl)-methanones. Sequential bioisosteric replacements of the arene rings of the indole led to the discovery of benzimidazole and thienopyrrole piperazinyl carboxamides as antagonists of the H_4 receptor. While attempting to synthesize the aforementioned benzimidazole carboxamides, an alternate series containing a quinoxaline 2-one core was also found have potency at the histamine H_4 receptor. The synthesis of these series and SAR derived from substitution around the arene rings will be discussed.

[34] O

Exploration of structure activity relationships for dual H_3 antagonists/serotonin transporter reuptake inhibitors

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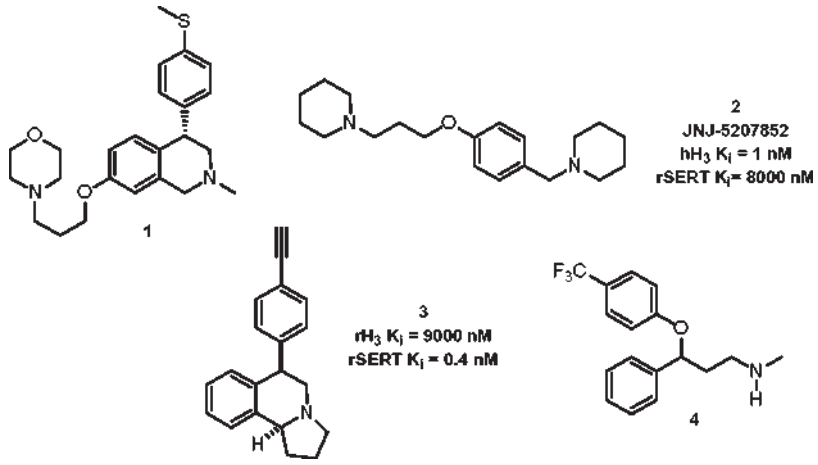
We have previously described a series of isoquinolines (e.g. 1) that are potent histamine H_3 antagonists and serotonin reuptake transporter inhibitors (1,2). These initial approaches relied on the incorporation of an "H_3-pharmacophore" into a fragment of the hexahydropyrroloisoquinoline core of 3 (3). More recently we have further explored the structure activity relationships around 3 and also examined the known antidepressant 4 as a potential template for dual activity compounds. These endeavors have led to the identification of several series of compounds with histamine H_3 antagonist activity together with activities at a range of monoamine transporters. Early results with respect to the structural requirements for the different biological targets will be presented together with preliminary in vivo pharmacological and pharmacokinetic data.



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[35] O

Optimization of H_3 antagonist series for H_3 selectivity over the hERG channel

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In some early series of histamine H_3 receptor antagonists, highly potent compounds were found to potently inhibit the hERG K+ channel, a potentially significant cardiovascular liability, as this channel is implicated in QTc prolongation. For example, benzofuran ABT-239 inhibited the channel (hERG Ki 195 nM, H_3R 0.45 nM) in a competition binding assay (versus ^{3}H-dofetilide). In the course of optimization to increase the selectivity, it was found that systematic modification of a specific structural region common to all the series was able to greatly reduce interaction with the hERG channel while retaining nM potency at the histamine H_3 receptor. For example, benzofuran (R)-4-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)-1H-pyrazole was weak at hERG (Ki >9000 nM). Similar findings applied to two newer series based on naphthylene and phenylcyclobutane core moieties, for example 4-{6-[2-((R)-2-Methyl-pyrrolidin-1-yl)-ethyl]-naphthalen-2-yl}-1H-pyrazole (hERG Ki >10,000 nM) and 5-{2-Fluoro-4-[3-((R)-2-methyl-pyrrolidin-1-yl)-cyclobutyl]-phenyl}-2-methoxy-pyrimidine (hERG Ki 3750 nM). The utility of the overall methodology was validated by selecting members of the improved series for more testing, where it was found that the new analgos retained the good drug likeness of earlier series (e.g. ABT-239), having good receptor selectivity, CNS penetration, and moderate plasma protein binding.

[36] O

Effect of the H_4R antagonist JNJ7777120 on the histamine content in the rat conjunctiva: a preliminary in vivo study

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The histamine (HI) H_4 receptor (H4R) has been shown to modulate immune system responses, thus expanding the role of HI in immunopharmacology and highlighting the therapeutic potential of H_4R antagonists in related conditions. We investigated, for the first time, the effects of the H_4R antagonist JNJ7777120 in a rat model of non-immunological conjunctivitis. Male Wistar rats of 200-250g were maintained under controlled conditions. JNJ7777120 (0.005-1mM), C48/80 (100mg/ml), cromolyn (CRM, 40mg/ml) were dissolved in normal saline. A 10μl drop of the drugs was applied topically into the lower conjunctival fornix of one eye, while the controlateral eye served as the respective control followed by sacrifice of the animals after 20min (licence no K/6017/06). The conjunctivae were removed and HI levels were determined fluorometrically. JNJ7777120 induced dose-dependent increases in the HI content ($r=0.814$, $p<0.001$). Coadministration with C48/80 dose-dependently reduced the effect of the H_4R antagonist, while coadministration with CRM shifted the dose-response curve to the right in a dose-dependent manner ($p<0.05$). Co-administration of JNJ7777120, C48/80 and CRM resulted in partial dosedependent alterations of the H_4R antagonist effect. The increases induced by JNJ7777120 could be attributed to altered HI metabolism, decreased basal HI release via stabilisation of mast cells or mobilisation/accumulation of the amine via transport from other sources. The decreased HI content, after challenge with C48/80 may imply an immediate HI releasing action, followed by rapid removal of the amine from the ocular tissues. The inhibitory effect of C48/80 on the action of JNJ7777120 indicated a biphasic antagonistic effect, the C48/80 action being more potent at high concentrations of JNJ7777120, while at lower concentrations, the blocker was more effective in inhibiting C48/80-induced degranulation. The parallel shift to the right of the JNJ7777120 dose-response curve by CRM and the effects of the JNJ7777120, C48/80 and CRM combination may indicate a competitive/allosteric action of JNJ7777120 and CRM on the H_4R.

[37] O

Histamine interferes with the insulin-like growth factor 2 receptor (Igf2R) - fibulin-5 (Fbln5) axis via H_3R in mouse melanoma

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In our previous work we generated three novel, transgenic mouse B16-F10 melanoma subclones with different capacities of histamine secretion. Using this model, we demonstrated that histamine is able to enhance melanoma progression in C57Bl/6 mice, as it supports both experimental tumor growth and metastasis formation. In the present study, we applied high throughput, global genome gene expression profiling to comprehensively describe the pathways related to histamine action in this model. Gene expression profiles of experimental murine melanomas, secreting different amounts of histamine, were compared by 44k mouse whole genome expression microarrays. Initial results were confirmed by real time PCR, and genes behaving histamine-coupled were further checked by immunohistochemistry, analyzing tumor tissue sections. Next, we tested the effect of the H_1 and H_2 receptor antagonists' loratadine and famotidine, respectively, administered orally to the animals, on tumor growth rate and expression levels of the previously identified histamine-coupled genes. Finally, an attempt was made to integrate the observations in functional gene pathways by mining gene network databases. Our results show that in contrast to earlier suggestions, the tumor growth-supporting effect of histamine is rather H_1R-, than H_2R-coupled. Histamine enhances expression of the DNA replication licensing factor CDT/1, and that of the asparagine synthetase, which is very interesting because many current chemotherapies target the enhanced asparagine metabolism of tumor cells. Further, histamine suppresses the expression of the well-known tumor suppressor insulin like growth factor 2 receptor (Igf2R) and that of the anti-angiogenic extracellular matrix protein fibulin-5 (Fbln5) via H_1R. Gene network analysis suggests that the latter two genes may be functionally coupled, because Igf2R is needed to activate matrix-bound pro-TGFbeta, a factor known to be required to stabilize Fbln5 expression. To our knowledge this is the first report comprehensively describing the effects of histamine on a tumor by global gene expression profiling. Microarray data suggests that histamine affects many vital aspects of tumor progression, such as proliferation, angiogenesis and even amino acid metabolism of melanoma cells.

[38] O

Regulation of neurotrophin synthesis in astrocytes by histamine and other monoamines

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Astrocytes as an active part of the tripartite synapse control neuronal activity and synaptic transmission. Besides that they provide trophic assistance to neurons by producing various neurotrophic factors. Our previous studies showed that astrocytes synthesize and secrete a group of neurotrophic factors - neurotrophins nerve growth factor (NGF) [1], brain-derived neurotrophic factor (BDNF) [2] and neurotrophin-3 (NT-3). Their synthesis is susceptible to the stimulation by growth factors, neurotransmitters, drugs, cytokines [3, 4]. Since the synthesis of neurotrophins in astrocytes can respond to the synaptically released neurotransmitters, we focused our study on the influence of monoamines: histamine (HA), serotonin (5-HT), noradrenaline (NA), and dopamine (DA) on BDNF, NGF and NT-3 protein levels in cultured neonatal rat cortical astrocytes. The examined monoaminergic neurotransmitters showed diverse regulatory effects on the cellular content of neurotrophins, their maximal effects were dose- and time-dependent. HA (1 μM; 24 h) increased NGF cellular content by a 2.1-fold whereas it exerted only a minor stimulatory effect on BDNF and NT-3 synthesis. NGF levels were also effected by NA (1 μM) causing a 2-fold increase after 12 h and maximal, a 2.7-fold increase after 36 h of incubation. BDNF protein content was elevated by NA (1 μM; 6 h) causing a 4-fold increase, and by 5-HT (1 μM; 4 h) and DA (150 μM; 4 h) causing a 2.3-fold and a 2.2-fold increase in BDNF cellular level. NA (1 μM; 6 h) and DA (10 μM; 6 h) also potently increased NT-3 content (a 1.4-fold and a 2-fold, respectively). All neurotransmitters showed stimulatory but very specific and selective action on the neurotrophin synthesis. Our results confirm the involvement of monoaminergic systems in the regulation of the synthesis of neurotrophins in astrocytes and suggest the existence of a positive interaction between monoaminergic neuronal activity and astrocytic neurotrophic support in neuron-astrocyte crosstalk.

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[39] O

Histamine pivotal role in regulating mammary carcinogenesis

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We have previously reported that HA modulates proliferation of MDA-MB-231 breast cancer cells in a dose-dependent manner. The effect of 10 mM HA was associated with an induction of cell cycle arrest, differentiation and apoptosis. In the present work we analyzed the gene expression profiles using expression macroarray analysis and identified several genes in the angiogenic and survival pathways that are modulated by 10 mM HA in MDA-MB-231 cells.

HA markedly up-regulated expression of genes related to growth inhibition and apoptosis as INFβ; CDKN1C (p57); casein β; IL-4; WISP1 (25, 40, 29, 65, 83-fold, respectively). INFα,γ, BCL-XS, IL-4R, TNFSF10, PPARγ, seemed to increase but to a minor extent. Conversely, HA increased expression of genes linked to migration and angiogenesis like ADAMTS1,8; RNASE4; EDG1; ETS-1; FGF1,2,7; ITGB3; PDGFRB; THBS2; TGFA; COL1A1 (41, 84, 31, 42, 265, 9, 33, 40, 9, 24, 14, 30, 52-fold) while decreased the expression of TGFB3; TGFR1,2; TIMP-1; VEGFB. In addition, we investigated whether H_3R and H_4R were implicated in biological responses triggered by HA. Employing specific HA receptor agonists and antagonists we demonstrated that the positive effect on proliferation was exerted via the H_3R while the decrease in proliferation was mediated via the H_1R, H_2R, and H_4R. By using Transwell system we determined that HA induced MDA-MB-231 cell migration. This effect was mimicked by Imetit while was inhibited by Clobenpropit, suggesting that HA could stimulate cell migration via H_3R playing an important role in invasion and metastasis.

Present study, describes a dual role for histamine in tumorigenesis acting not only anti-oncogenically by reducing proliferation and increasing apoptosis and differentiation but also pro-oncogenically by stimulating proliferation and cell migration. It also suggests that H_3R may be involved in the regulation of breast carcinogenesis representing a novel molecular target for new therapeutic approach.

[40] O

Comparative investigations of the influence of H_1-antihistamines on the generation of the reactive oxygen species by phagocytes

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H_1-antihistamines are the most widely used drugs suppressing histaminergic allergic reactions, however, their effects on phagocytes are not fully understood. Inhibitory effects of four selected H_1-antihistamines on oxidative burst of phagocytes were found in our previous study [1]. Herein, ability of acrivastine, antazolin-hydrochloride, astemizol, bromadryl, brompheniramine-maleate, clemastine-fumarate, cyclizine-hydrochloride, dithiaden, chlorcyclizine-hydrochloride, chlorpheniramine-maleate, ketotifen fumarate, loratadine, oxatomid and pheniramine-maleate (in a concentration range of 0.5 - 0.001 mmol/l) to modulate production of reactive oxygen species (ROS) by phagocytes, to modulate myeloperoxidase (MPO) activity and their antioxidative properties were evaluated. The production of ROS by rat phagocytes was analysed by luminol-enhanced chemiluminescence. The activity of MPO and scavenging effects of drugs against peroxyl, hydroxyl and superoxide anion radicals were evaluated luminometrically, fluorometrically and spectrophotometrically. All tested H_1-antihistamines, except acrivastine, inhibited phagocyte ROS production in a dose dependent manner. The strongest inhibitory effect revealed astemizol, clemastine-fumarate and chlorcyclizine-hydrochloride. Tested H_1-antihistamines scavenged peroxyl radical (most significantly astemizole, bromadryl and dithiaden) while none of the tested drugs scavenged superoxide and hydroxyl radicals in cell free systems. A significant decrease in MPO peroxidation activity was observed in the presence of astemizol anddithiaden. Our results suggest that the inhibitory effects of selected H_1-antihistamines (e.g. astemizol, dithiaden) on ROS production by phagocytes can be explained by their effects on MPO activity as well as by their direct antioxidative properties.

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[41] O

Pharmacological actions of a new synthetic cyclodepsipeptide, the A83586C-citropeptin hybrid, *v.* complement C5a

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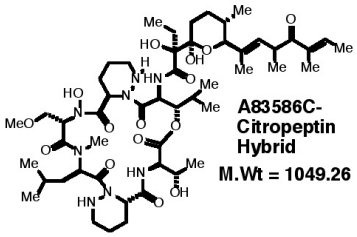

The complement anaphylatoxin C5a can stimulate smooth muscle contraction and the release of histamine (from mast cells) and oxidative products [1,2]. It has been reported that C5a can act via G-protein transduction pathways [3]. Aurantimycin-A (AA) is a natural fungal antibiotic (peptide in nature) with C5a antagonist activity which we have tested, along with a new structurally-related A83586C-Citropeptin (AC) hybrid (obtained via total synthesis)[4], in guinea-pig trachea (GPT)[5] and rat ileum (RI)[6] smooth muscle, and in rat peritoneal mast cells (RPMC). In GPT both AA & AC (10-30 μM) were very weak partial agonists by themselves, and 20 min preincubation appeared to selectively block the weak contraction produced by human C5a (65-87 C-terminal, Bachem; E. coli recombinant human C5a, Sigma, is being tested). With longer preincubation (1-4 h) AA and AC additionally blocked contraction by other agonists (histamine, 5-hydroxytryptamine & carbachol, tested so far) in a concentration and preincubation time-dependent manner, but was non-competitive, reversible, and relatively differential, e.g. it inhibited histamine- and 5-hydroxytryptamine-induced contraction to a greater extent than carbachol. In RI AC behaved as an antagonist as well as a partial agonist (AA is being tested). AC blocked contraction induced by C5a or H_2O_2, but not that by the free radical generator 2-2′ azobis (2-aminopropane)(AAHP). Benzalkonium chloride (BCl, G-protein inhibitor) blocked the contraction produced by C5a or H_2O_2, but not that by AAHP. In RPMC AC induced histamine release in a time-, pH-, temperature- and Ca^{2+}-dependent manner, was not cytotoxic, and was inhibited by BCl. The present results suggest that AA and AC, in addition to the probably competitive inhibition of C5a-induced contraction of GPT with short preincubation, after long incubation, also inhibit the contraction to other agonists non-competitively, perhaps through the Gαq-coupling pathway. The RI experiments suggest that AC might have an additional anti-oxidant effect. It is interesting that the contraction of RI and RPMC histamine release induced by AC were reduced by BCl, indicating the involvement of G-protein in both processes.

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[42] O

Mastication and oral load of L-histidine induce efficient reduction of visceral adiposity through activation of histamine neurons

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Visceral adiposity is an essential component of metabolic syndrome. Reduction of excessive visceral fat prevents metabolic syndrome and improves fatal atherosclerotic diseases. The present topics aim to demonstrate that activation of histamine neurons driven by mastication and/or L-histidine is useful for selective improvement of visceral adiposity. Moderately Japanese obese patients, 23-67 years old, together with inborn obese model animals were used. Depletion of neuronal histamine from the mastication center (Me5), reduced eating speed, but that from the satiety center (VMH), increased both meal size and its duration leaving eating speed unaffected. Neuronal histamine turnover in the Me5 elevated at the early phase of feeding, but that in the VMH did at the later phase. The elevation of turnover was abolished by gastric intubations of an isocaloric liquid diet or an equivolume of water. Mastication-induced activation of histamine neurons suppressed physiological food intake through H_1-receptor in the hypothalamic paraventricular nucleus (PVN) and the VMH. Simultaneously, the activation of the PVN accelerated lipolysis predominantly in the visceral adipose tissues and up-regulated mRNA expression of uncoupling proteins through sympathetic efferent nerve. Mastication thus plays an important role as a potent input signal to energy metabolism through activation of histamine neurons. In fact, mastication before meals improved visceral adiposity in obese patients. According our results, histamine neuron systems make a negative feedback loop tightly with leptin signaling system. Based on our series of histamine study, L-histidine was as well effective as brain food because its oral load to rats activated hypothalamic histamine neurons efficiently. Practice of mastication at each meal together with boosted consumption of foods rich in L-histidine was useful for improvement of and prevention from visceral fat accumulation and morbid obesity.

[43] O

Comparison of the effect of 4-methyl histamine on human and mouse activated basophils

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Background: Histamine and methyl-4 histamine are known to induce a negative feed back on human and mice activated basophils via H_2 receptors. We compared the activity of 4-methyl histamine on human and mouse activated basophils.
Methods: Human basophils were obtained by buffy coat and mouse basophils were obtained from bone marrow and cultured in the presence of IL_3. Human basophils were stimulated by the formyl Methyl Leucine Peptide (fMLP, 10^{-7}M) and mouse basophils both by IgE (1µg/ml) and by IL_3 (5ng/ml). Human basophil activation was measured by flow cytometry using the IgE/CD203c protocol and results were expressed in activation indexes and mouse basophil activation was measured by histamine and IL_4 release and results expressed in amount of mediator released. The 4-methyl histamine was tested at 10-3M.
Results: On 10 successive experiments, fMLP induced human basophil activation was inhibited by 4-methyl histamine. This effect was significant on CD203c expression (86.5%, p=0.003) and not on IgE down regulation. On mouse basophils, two successive experiments showed that 4-methyl histamine inhibited both IgE and IL_3 induced IL_4 release (respectively 88% and 98%and 92% and 96%). Three other experiments for which 4-methyl histamine was tested within the concentration range of 100mM to 100nM showed that 4-methyl histamine inhibited IL_3 induced IL_4 release from mouse basophils from 1 mM to 1 µM
Conclusions: We confirm here by flow cytometry and IL4 release that 4-methyl histamine, H_2 agonist, induced a potent negative feed back of human and mouse.

[44] O

Leucotriene measurements for the diagnosis of peranaesthetic allergic events

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Skin tests are currently used to differentiate between allergic and nonallergic immediate hypersensitivity. However the reliability of skin tests with anaesthetic agents is discussed. Biochemical tests are recommended : histamine concentration increases reflect specific or non-specific release, whereas tryptase increases favor a specific mechanism. Leukotrienes are synthesized as a response to antigen-IgE binding and could be a specific marker of allergic events. We measured leukotrienes C_4, D_4, E_4 with ELISA (Cell Com, Immunotech) in urines collected from 17 volunteer controls before and after general anaesthesia and surgery, and retrospectively in 4 patients who had severely reacted during anaesthesia. Results were expressed as a ratio to creatinine concentration. In the controls, the mean leukotriene concentrations ± standard deviation were respectively 75±38 µg per mole creatinine before the procedure and 98±43 µg/mol after. The difference was significant (paired t-test, $p<0.006$). The four patients had increased concentrations of histamine (RIA, Immunotech) and tryptase (FIA, UniCAP, Phadia), the ranges were respectively 39-3940 nmol/L for histamine (normals < 6 nmol/l) and 37-49 µg/L for tryptase (normals < 12 µg/L). All of them had urinary leukotriene concentrations exceeding 184 µg/mol (mean + 2 S.D. of values after procedure), the individual values being : 4560 ; > 439 ; 204 ; 376 µg/mol. These findings show that surgical/anaesthetic procedures lead to a mild increase of leucotriene excretion. However concentrations are more widely increased after reactions with proved mast cell degranulation.

[45] O

Suppression of histamine H_1 receptor mRNA elevation in nasal mucosa of allergy model rats by prolonged pretreatment of antihistamines

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Antihistamines are major therapeutics for pollinosis. Pretreatment of antihistamines is currently recommended to avoid severe symptoms. However, scientific basis of the pretreatment has not been elucidated. Elevation of histamine H_1 receptor (H_1R) and interleukin-4 (IL-4) mRNA was observed in the nasal mucosa of allergy model rats. This study was carried out to explore the possibility that long-term pretreatment with antihistamine could strongly prevent symptom and elevation of mRNA in allergy model rat. Allergy model rats were prepared by sensitizing 6 weeks old Brown Norway rats by exposure to toluene 2,4-diisocyanate (TDI). Antihistamines were administered 1 hour prior to TDI sensitization for different duration of time. Nasal allergy like behavior was scored during a 10 minutes period just after provocation and mRNA was determined by quantitative real time RT-PCR. Single and three day treatment with epinastine only partially suppressed sneezing, H_1R and IL-4 mRNA elevation in nasal mucosa of TDI sensitized rat. However, administration of epinastine for 1, 3 and 5 weeks prior to provocation showed strong suppression of sneezing and mRNA elevation. Olopatadine and d-chlorpeniramine showed similar profile. IL-4 is an allergy cytokine. Application of IL-4 in nasal cavity of rats induces a time and dose dependent increase in H_1R mRNA expression in nasal mucosa. Result of this study showed that suppression of expression of H_1R and IL-4 mRNA could play a key role in symptom suppression by pretreatment for more than 1 week with antihistamine in addition to H_1R antagonism.

Poster presentation

[1] P

Human beta-defensin 3 expression and mean histamine concentration-human placental tissue study

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Human defensins are small cationic, cysteine-rich peptides with a wide range of antimicrobial activities. Human beta-defensin 3 (hBD-3) is widely expressed in placenta and exhibits antimicrobial activity against a number of human pathogens, including multiresistant Gram-postitive bacteria Staphylococcus aureus and vancomycin-resistant Enterococcus faecium as well as Gram-negative (Pseudomonas aeruginosa, Escherichia coli) and yeast Candida albicans. Stimulated by infection of bacteria and by interferon gamma, amnion and placental cells produce hBD-3. Considering, that hBD-3 induces histamine (HA) release from mast cells, we examined correlation between hBD-3 expression and mean HA concentration in normal (N =12; Group II) and chorioamnionitis-complicated pregnancies (N=12; Group I). The gravidas in both groups were gestationally matched. HA concentrations were estimated fluorimetrically in cuts collected from the fetal surface of the placenta in standardized manner. The same sample collection protocol was used for the study of hBD-3 expression. After immunostaining, hBD-3 expression was examined in 5 μm paraffin sections by computed morphometry for quantitative analysis. The hBD-3 expression was significantly +/-SEM) of the value calculated in Group II (controls) taken as 100%. Mean HA concentration within placental tissue was negatively correlated ($p<0.05$) with hBD-3 expression (ng per gram of wet tissue, mean +/-SEM: 109.15 +/-11.07 versus 142.32 +/-12.83; groups I and II, respectively). We concluded, that studying antimicrobial role of hBD-3 in vivo, or possible use of defensins as a new class of antibiotics, we should also take into consideration degranulating properties of these compounds.

[2] P

Increased production of human beta-defensin 3 (hBD-3) by human amniotic epithelial cells (HAEC) after activation of toll-like receptor 4 (TLR4) in chorioamnionitis

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Placenta and fetal membranes may become exposed to pathogenic micro-organisms, such as bacteria and viruses, which may influence the embryo/fetus well-being. The results of recent investigations suggest, that placental unit may function as an active barrier, recognizing and responding to pathogens via toll-like receptors (TLRs). The TLR4 recognize lipopolysaccharide (LPS), the microbial product associated with gram-negative bacteria. Various antimicrobial molecules are expressed not only on the surfaces of monocytes but also on epithelial cells. HAEC have the ability to produce antimicrobial beta-defensins, especially hBD-3. We examined comparatively in vitro, influence of LPS on hBD-3 synthesis in normal HAEC (Group II; N=12) and obtained after chorioamnionitis (Group I; N=12). The presence of TLR4 was confirmed by immunohistochemistry in 5 paraffin sections of human fetal membranes from pregnancies with or without histologic chorioamnionitis. HAEC were isolated from the amnion after deliveries (including cesarean sections) in term pregnancy and cultured in 24-well culture plate inserts (1.0 million cells per well) in Ham's F12 and Dulbeco's modified Eagle medium supplemented with 10% fetal calf serum. HAEC from wells were exposed to LPS (1 microg/ml) and after 24 hours the cultures were terminated. Concentrations of hBD-3 in culture media separated from HAEC were estimated in both groups using sandwich ELISA. Respective controls without LPS were also established. The concentration of hBD-3 was significantly increased in Group I, compared to the control Group II (373 +/-43 versus 181 +/-22; ng/ml, mean+/-SEM; $p<0.02$). The results indicate, that LPS-induced activation of TLR4 in chorioamnionitis triggers higher production of hBD-3. The hBD-3 appears to be the predominant epithelial defensin within the amnion. Considering its pro-inflammatory activity (e.g. induction of histamine release), this may explain why chorioamnionitis is often complicated by premature rupture of membranes.

[3] P

Does histamine influence differentiation of trophoblast in preeclampsia?

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Preeclampsia (PE) which is a relevant reason of complications of pregnancy seems to be a result of impaired throphoblast invasion. The complicated process of spiral arteries infiltration and replacement of their endothelium with trophoblast is parallel to change in trophoblast surface adhesion molecules. The cells of invasive trophoblast become alpha6-beta4 integrin negative and alpha5-beta1, alpha1-beta1 and alphav-beta3 integrins positive. It is reported that PE is associated with decreased expression of the alphaV-beta3 integrin. Our previous study showed enhancement of alphaV-beta3 integrin expression in trophoblast cell culture treated with histamine. The aim of this study was to examine the potency of histamine influence on alphaV-beta3 integrin expression in trophoblast derived from preeclamptic placentae. Placentae were collected from normal (n=7; group 1) and PE-complicated pregnancies (n=8; group 2) were obtained after term deliveries. Trophoblast cell cultures were established using modified Kliman's method. Histamine alone or with: pyrilamine, cimetidine, clobenpropide or NaCl(0,9%) were added daily into the vessels (culture H, P, C, CB and control, respectively). After 48, 72 and 96 hours, cells were detached from vessels and expression of integrin alphaV-beta3 was measured with ELISA. The expression of integrin alphaV-beta3 is given in percentage of that observed in control culture from group 1. The highest levels of alphav-beta3 expression were observed in culture H, C, and CB, group 1 after 72 hours: H 45%±13.3, C 48% ±15.5, CB 35.4%±12.2, $p<0.05$ and 96 hours: H 67.8%±12.2, C 35.2%±18.2, CB 47%±6.7, $p<0.05$. In culture P there were no siginificant difference at any hours of culture. More we did not observe increased expression of alphav-beta3 integrin in any culture of group 2.
Histamine stimulates alphaV-beta3 expression in trophoblast cells culture derived from uncomplicated pregnancies acting through H_1 receptor but not in preeclamptic placentae.

[4] P

Microvesicles: a new immunological communication pathway between the fetus and the mother. Sudies in asthmatic and healthy pregnancies

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Microvesicles (Mv) are membrane covered small particles, derived from various cell types. Their possible diverse origine includes, among others, blood cells and even trophoblast cells. It is a relatively new finding that Mv-s play an important role in the information transfer between different cell types, so Mv-s are involved in cell to cell crosstalk as cellular effectors. Successful pregnancy requires a series of interactions between the maternal immune system and the implanted fetus. As we have already shown in our earlier studies that histamine influences pregnancy-related processes, we focused on this new field, in order to investigate the potential role of Mv-s in the physiology of pregnancy.
Aims:-To identify the cellular origine of Mv-s. (multicolor flow cytometry, confocal microscope). -To characterize the functional role of circulating Mv-s. (treatment of lymphocytes by ultracentrifugation-isolated Mv-s, proliferation assays).
Results: 1. We proved the existence of Mv-s and isolated them from the serum of mice and humans, both asthmatic and healthy, in pregnant and non-pregnant states. 2. We found a decreased number of thrombocyte-derived Mv-s, meanwhile an increased ratio of lymphocyte/trophoblast derived Mv-s in healthy human pregnants. Oppository, in asthmatic pregnants this alteration could not be detected. 3. Mv-s, isolated from pregnant plasma, bind to the cells of the immune system with higher affinity. 4. We found difference between lymphocyte proliferation-inducing effect of pregnancy derived Mv-s. We can conclude that Mv-s do play a role in the information flow between different cell types during pregnancy, moreover, in a very specific way.

[5] P

Histamine increase the invasive potential of human melanoma cells through H_1 and H_2 receptors

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The incidence of malignant melanoma was significantly increased recent years. The patients with the disease exhibit reduced life expectancy. Histamine is an important paracrine and autocrine regulator of normal and tumor cell proliferation and differentation. Melanoma cells reveal autonomous histamine metabolism. We demonstrate the four histamine-receptors and the investigated invasion markers on the sections of patients with melanoma. Our aims were to investigate the effects of histamine on the migration, expression of adhesion molecules, chemokines, matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF-C) in two human melanoma cell lines with different invasive potential. Modified Boyden-chamber technique was applied to evaluate the effects of histamine on the migration. Immunocytochemistry, flow cytometry and real-time RT-PCR were carried out to estimate correctly the changes of the expression of invasion markers following histamine treatments with different concentration and administration time. We found that histamine had chemorepellent effect on both melanoma cell lines. Expression of CXCR-4, CXCL-12, MMP-2, MMP-3 and VEGF-C were upregulated, while CD44, NCAM and ICAM-1 were downregulated upon the histamine treatments. Histamine plays a key role in several steps of the metastatic cascade such as migration, adhesion and invasion. These effects are mediated through H1 and H2 receptors. Our in vitro results suggest that histamine decreases directly the migration and adhesion ability of human melanoma cells. On the other hand, histamine indirectly increases the invasive potential of melanoma cells by upregulating CXCR-4, CXCL-12, MMP-2, MMP-3 and VEGF-C.

[6] P

Characterization of the effect of histamine in mice corpus cavernosum

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The use of H_2-antagonists for the treatment of peptic ulcer disease has been associated with reports of erectile failure. Isolated human corpus cavernosum tissue studied in vitro shows indeed a relaxing influence of histamine and injections of histamine intracavernously in human cause erection in all patients. These functional observations and the fact that histamine-containing mastocytes have been identified in erectile tissue indicate that histamine may play a role in human penile erection. However, the mechanisms involved seem to be species-dependent. As mice cavernosal tissue is proposed as a good model for human tissue, the present study aimed to found out some characteristics of the effect of histamine on this tissue. The study was performed using an in vitro organ bath technique for measuring isometric tension changes of isolated corpora cavernosa using a Krebs-Ringer bicarbonate solution bubbled with 95% O2-5%CO2 at 37°C. At basal resting tension, the addition of histamine had no influence on tone. On precontracted (norepinephrine 5µM) preparations histamine elicits a strong relaxing effect (pEC50 = 4.78 + 0.13;Emax = 86.35+ 3.25; n=19). The concentration-relaxationcurve of histamine is shifted to the right (without inhibition of Emax) in the presence of H_2-receptor antagonist cimetidine (0.1 mM),but not in the presence of H_1-receptor antagonist pyrilamine (10 µM). In preparations pretreated with cimetidine, the additional presence of pyrilamine or the H_3-receptor antagonist thioperamide (10 µM) did not further inhibit the relaxing effect of histamine. The presence of histamine (10 µM) did not influence the relaxing effect elicited by electrical field stimulation. Addition of H_2-agonist dimaprit elicited a substantial relaxation of precontracted preparations. It is concluded that histamine elicits a strong relaxation of mice corpus cavernosum by activation of H_2-receptors and does not interfere with mice cavernosal nervous erectile activity in vitro.

[7] P

Down regulation of histamine receptors in human colon cancer

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Four histamine receptors mediate the variable histamine effects on different cells. Normal human gastrointestinnal tract contains H_1, H_2, H_4 but not the H_3 histamine receptors. Our aims was to compare the histamine receptor expression of normal colon samples to the receptor expression of colon cancer patients. Normal and tumor samples of 40 patients (10-10 from each Dukes stages: A, B, C and D) were used to check to histamine receptor expression using real time PCR, Western blot analysis and immunohistochemistry. All of the normal and tumor samples we could detect the expression of H_1 and H_2 receptors. H_4 receptor was present in more then 90 % of the samples, while only 2-3 samples had H_3 receptor expression by real time PCR. Similar results were found using Western blot analysis of the samples. Immunohistochemical examination reveals, that not only normal enterocytes but the tumor cells were immunopositive cells as well for H_1, H_2 and H_4 receptors. Compare to the normal and tumor samples of the same patiens it was found that in all of the Dukes stages H_1 and H_4 receptor expression level is significantly lower than in the normal tissue while the H_2 receptor expression is unchanged. These results show, that the histamine receptor balance is changed in all Dukes stages of colon tumor patients. Moreover our results support that fact, that histamine through its receptors is involved in the tumor progression. It is known that histamine through H_2 receptor could be a growth factor not only for melanoma but for the colon cancer too. So unchanged H_2 receptor with a decreased H_1 and H_4 receptor level could be a very favorable for the tumor progression.

[8] P

Famotidine, a H_2 receptor antagonist, decreases the late phase of orthodontic tooth movement in rats

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Remodelling of the alveolar bone and the periodontal ligament (PDL) is the key process in orthodontic tooth movement. It consists of tissue formation and resorption. On the pressure side of the loaded tooth resorption of the alveolar bone and the PDL takes place. On the tension side new bone is formed and the PDL remains intact. Many chemical messengers are involved in this process. Histamine is one of them.
The aim of the study is to determine the influence of H_2 receptors antagonist famotidine on orthodontic tooth movement.
The study was performed on 30 male Wistar rats (320-330 g), divided into 3 groups (n=10). Animals from groups I and II were applied a superelastic tractive coil spring (F=25g) between the upper left first molar and upper left incisor. Group I was daily treated with famotidine (10 mg/kg) and group II with a placebo. Group III was treated with a placebo. Measurements between the teeth were taken with a digitronic caliper (precision 0.01 mm) on days 0, 7, 14, 21, 28, 35 and 42.
The distance between the upper left first molar and the upper left incisor decreased in all appliance groups. In the first group of animals treated daily with famotidine tooth movement was significantly less ($p<0.001$) on days 35 and 42 when compared to the second group of animals. In the third group of animals the distance between the upper left first molar and the upper left incisor increased during the study, because of the natural distal drift of the molars.
Many researchers have shown that there are several phases of orthodontic tooth movement. The first phase lasts up to 2 days and represents the movement of the tooth inside its alveola. The second phase, which lasts for 20-30 days, represents the time, when almost nor only little tooth movement takes place. After that the third phase starts. It represents actual tooth movement through bone.
Histamine influences the late phase of orthodontic tooth movement in rats via H_2 receptors.

[9] P

Cetirizine, H_3 receptor antagonist, decreases the first stage of orthodontic tooth movement in rats

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During orthodontic tooth movement remodeling of dental and paradental tissues occurs involving the alveolar bone, periodontal ligament (PDL), dental pulp and gingiva. Orthodontic forces applied to the tooth alter the vascularity and blood flow of the PDL, resulting in release of various chemical mediators in the paradental tissue. The actual involvement of histamine in orthodontic tooth movement has not been established yet. The aim of the present study was to determine the role of a selective H1 receptor antagonist cetirizine on orthodontic tooth movement in rats.
Thirty adult male Wistar rats were divided into three groups (N=10). In Group I a superelastic closed coil spring (F=25cN) was used and the animals were treated daily with 10 mg/kg body weight of cetirizine. In Group II a closed coil spring was also used and the animals were treated with a placebo daily. Animals in Group III were treated with a placebo daily. The coil spring was attached between the upper left first molar and the upper left incisor. The distance between the teeth was measured with a digital calliper on days 0, 7, 14, 21, 28, 35 and 42.
Tooth movement was significantly less on day 7 in animals in Group I which were treated with cetirizine ($p<0.05$) compared to animals in Group II. There was no significant difference in tooth movement between animals in Group I and those in Group II from day 14 to day 42 of the experiment. In group III the measured distance increased during the entire duration of the experiment due to natural distal drift of the molars ($p<0.001$). According to the results the action of histamine plays a significant role only in the initial phase of orthodontic tooth movement. This phase coincides with acute inflammation of paradental tissues in the first stage of orthodontic tooth movement. The later stages are not significantly affected.
Histamine influences the initial phase of orthodontic tooth movement in rats via H_1 receptors.

[10] P

Histamine releasing factor (HRF) in pannus of joints affected by rheumatoid arthritis

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Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes chronic inflammation of connective tissue, primarily in the joints. The histaminic pathway and histamine receptors participate in the pathogenesis of RA but an implication of histamine-releasing factor (HRF) in the disease was not studied. RA affects the synovial membrane, articular cartilage, fibrous joint capsule, and surrounding ligaments and tendons. Over denuded areas of the synovial membrane, the granulation tissue called pannus develops. The function of pannus is not known and whether pannus is a cause or an effect of articular cartilage involvement in RA is not clear. In the present study we examine the ultrastructure, expression and localization of HRF mRNA and protein in pannus of joints affected by RA. Samples of knee cartilage from patients with RA were removed at joint replacement surgery. The study was approved by the local ethical committee (217/2000). Paraffin sections of pannus were used for in situ hybridization with riboprobs of human HRF and digoxigenin-labeled compounds. For immunohistochemistry polyclonal antibodies generated against different domains of HRF protein were produced by Santa Cruz Biotechnology (USA). Epon ultra-thin sections were used for study of pannus ultrastructure and subcellular localization HRF in pannus.
The results show that characteristic feature of pannus ultrastructure is the presence of syncitial tissue, numerous undifferentiated cells, and chondroblasts. In addition, fibroblasts, macrophages and chondrocytes were also found. The high expression of HRF mRNA and presence of HRF protein were localized mainly in syncitial tissue and in all immature cells. In the conclusion, we document for the first time that pannus of RA joints contains HRF protein that may be a new "target" protein for pharmaceutical studies and may contribute to a new strategy in RA treatment.

[11] P

Expression of histamine H_4 receptor in human osteoarthritic synovial tissue

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Degenerative joint disease (osteoarthritis OA) is the most prevalent noninflammatory joint disease. Recently histamine (H) has been implicated in the pathophysiological processes of OA and H receptors H_1 and H_2 as well as histamine decarboxylase expression were immunohistochemically demonstrated in OA chondrocytes. We have previously shown (Grzybowska-Kowalczyk A. Inflamm Res 2007 in press) that the novel HH_4 receptor is localized in synoviocytes of inflamed area of chronic inflammatory disease rheumatoid arthritis (RA). Here we report the studies of expression of HH_4 receptor in various types of synovium cells of patients with OA. Synovium samples were obtained at joint replacement surgery of OA patients or from age matched adults as controls. Samples were fixed in 4% paraformaldehyde and H_4R was localized by immunohistochemical staining using the primary polyclonal antibodies. We demonstrated the presence of H_4 protein in various types of synovial cells and the distribution was similar to observed in RA samples. These findings suggest that H is an important contributor to pathogenesis of OA also via its H_4 receptor and that the suitable antihistamine therapy could be considered.

[12] P

Cardiovascular reactivity to histamine in a highsodium intake model of preeclampsia in rats

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

[13] P

Effect of Histamine on cell proliferation, apoptotic death and cell differentiation in cultured cortical neural stem cells, on the way to study the histaminergic role during cerebral cortex development

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Histamine (HA) is one of the first neurotransmitters to appear in the developing vertebrate nervous system. In the adult, histaminergic neurons are localized exclusively in the tuberomammilary nucleus, but during central nervous system (CNS) development there are different groups of histaminergic cells. Between embryonic days (E) 13-18 in rat, immunoreactivity for HA and its forming enzyme L-histidine decarboxylase, can be detected in rhombencephalon, choroid plexus and the area adjacent to the third and fourth ventricles. Also, histaminergic fibers can be observed at E15 in the hypothalamus, frontal and parietal cortex. Furthermore, HA concentration is several-fold higher during development than in the mature CNS and this rise correlates in time with high rates of neurogenesis. Since CNS development involve different processes such as proliferation, differentiation and cell death, we performed in vitro experiments using E14 neuroepithelial stem cells from rat cerebral cortex (NSC, neural stem cells) to study the role of HA during CNS formation. Cells were cultured 4 days in N2 medium containing FGF2 followed by 6 days in N2 medium to permit differentiation. In order to know if NSC expresses HA receptors, RNA was isolated and by RT-PCR histaminergic receptors (H_1, H_2 and H_3) were amplified in both proliferative and differentiated cells. Treatments with increasing amounts of HA from 100 nM up to 1mM were applied and cell proliferation was measure by cresyl violet and BrdU incorporation assays, showing an increase in cell proliferation ranging from 19-37%. During proliferation HA had a protective effect, in contrast HA was capable to increase cell death after differentiation as measured by TUNEL. Neural markers were used to quantitatively measure the effects of HA during differentiation. The immunocytochemistry made after differentiation showed an increase in the number of MAP2-positive neurons from 9% to 21% and a decrease on the proportion of GFAP-positive glial cells from 37% to 22% when comparing control conditions with 1mM HA. Our data show that NSC express HA receptors and that HA regulates cell number during both proliferative and differentiative conditions and cell fate *in vitro*, which suggest that HA might play an important role during CNS development as a neuronal-inducing agent.
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[14] P

Histamine regulates pancreatic carcinoma cell growth through H_1, H_2, H_3 and H_4 receptors

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PANC-1 is a cell line derived from a human ductal pancreatic carcinoma. We have previously reported that histamine (HA) H_1 and H_2 receptors are expressed in these cells. Nanomolar HA doses stimulate cell proliferation while micromolar concentrations inhibit clonogenic growth. This inhibition is mediated by H_1 and H_2 receptors. We have also found out that intracellular levels of nitric oxide (NO) modulates PANC-1 cells proliferation and that the antiproliferative effect exerted by HA may be mediated by NO levels. In the present work we evaluated the presence of H_3 and H_4 receptors in PANC-1 cells and the action of HA on the growth of pancreatic carcinoma xenografts in nude mice. The expression of H_3 and H_4 receptors was demonstrated by immunoblotting and immunocitochemistry. Both receptors showed to be involved in cell proliferation when the respective agonists/antagonists were tested, being cell growth increased through H_3 receptors and diminished by H_4 receptors. Nude mice were sc inoculated with 3x106 PANC-1 cells. When tumor volume reached 65 mm3, animals received the following treatments during 25 days: HA (1mg/kg day, sc), aminoguanidine, a NO synthase inhibitor, (AG, po 2 or 4 mg/ml daily in drinking water), ranitidine (50 mg/kg day, po) or loratadine (Lor, 2.5 mg/ kg day, po). Tumor growth was significantly increased in HA and Lor treated animals while it was decreased in AG treated mice. The tumor expression of Bcl-2 family members determined by immunohistochemistry showed that Bcl-2/Bax ratio is enhanced by HA, Lor, and AG. Tumor vascularization assessment by Masson trichromic stain was augmented by HA but decreased by AG. In addition, immunohistochemical evaluation indicated that intratumoral levels of HA were higher in faster growing tumors In summary, HA stimulates pancreatic tumor cell proliferation in vivo exerting a direct antiapoptotic action. This effect on tumor growth is associated to an increased tumor synthesis of HA and to an angiogenic action.

[15] P

Histamine uptake into human vascular endothelial cells and influence of some antidepressant drugs

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Following its release, histamine rapidly disappears from circulation with $t_{1/2}$ of 0.5-2 min. This rapid inactivation of intravascular histamine can be due to either enzyme degradation within blood vessels or uptake into the endothelial cells. Some antidepressant drugs affect plasma levels of histamine. Since, amitriptyline and citalopram caused a rapid and significant decrease of histamine plasma levels after its application in rats (Irman-Florjanc et al, 2004), we were interested whether antidepressant drugs influence the uptake of histamine into endothelial cells. We used the model system of cultured human umbilical vascular endothelial cells (HUVEC) and followed ^{3}H-histamine uptake in a serum-free buffer. The amine was taken up by endothelial cells in a time-dependant manner from 5 to 45 minutes of incubation. Specific ^{3}H-histamine uptake followed Michaelis-Menten kinetics with a Km of 10 nM and a Vmax of 0.2 fmol/mg of protein/min. Further on, we studied the influence of 1 nM-1mM amitriptyline, desipramine and citalopram on ^{3}H-histamine uptake. Amitriptyline in the concentration range from 10 - 1000 nM significantly increased, whereas desipramine (0.001-0.01 mM) decreased, and in contrast, citalopram did not alter ^{3}H-histamine uptake into HUVEC. Amitriptyline with the lowest pKa value (pKa = 9.42) of antidepressant drugs tested increases, whereas desipramine with the highest pKa value (pKa = 10.44) inhibits the uptake of ^{3}H-histamine into HUVEC. We can conclude that endothelial cells actively participate in the inactivation process of histamine. The results indicate that antidepressant drugs can affect the transport of histamine into endothelial cells, but this effect may not be the main cause for previously observed rapid and significant decrease of plasma histamine after amitriptyline and citalopram treatment.

Irman-Florjanc et al. Inflamm Res 2004; 53: Suppl 1: S97-S98.

[16] P

The histaminergic system is a promising target for the treatment of leptin resistant obesity

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Leptin is a key signal linking peripheral adiposity level to the regulation of energy homeostasis in the brain. The injection of leptin decreases body weight and food intake in lean rodents, however, in rodent model of diet-induced obesity (DIO), the exogenous leptin cannot improve adiposity. This leptin ineffectiveness is called as leptin resistance, and the factors downstream of leptin signaling gather attention as a viable strategy to treat obesity. Several lines of evidence have proved that the histaminergic system controls feeding behavior, and we previously reported that the histaminergic system is one of the targets of leptin [1,2]. In the present study, the effects of leptin and the direct activation of the histaminergic system by the antagonism of H_3 receptors on hypothalamic histamine release and energy intake were investigated in normal and DIO mice.
Leptin (1.3mg/kg, ip) significantly increased hypothalamic histamine release and reduced 12h-energy intake in normal mice in accordance with our previous findings, but leptin affected neither histamine release nor energy intake in DIO mice. In contrast, clobenpropit (5mg/kg, ip) , an H_3-antagonist, elicited a significant increment of histamine release in both types of mice. Clobenpropit failed to decrease 12h-energy intake, however, it could decrease 3h-energy intake in both types of mice. These results suggest that the lack of the activation of the histaminergic system partly contributes to obesity in DIO mice, and that the direct activation of the histaminergic system is a promising strategy to circumvent leptin resistance obesity.

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[17] P

The free histidine pool formation and it's relationship with plasma nitric oxide level under chronic alcohol intoxication

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Background: Histidine is an immediate precursor of histamine. Therefore, the level of endogenous histidine may determine histamine secretion. Some effects of ethanol consumption may be connected with the endogenous histamine and nitric oxide level fluctuations. The data on influence of ethanol feeding on NO-synthase-system and histamine system are too small number and quite contradictory.
The aim of present investigation is to estimate the relationship between NO level and free histidine pool formation in chronic alcohol consumption conditions.
Methods: Experiments were performed on male Wistar rats (180g). I group fed ethanol in drug dose (3.5 g/kg, intragastrically, 25% solution) twofold per day during 42 days. II group fed ethanol as a same schedule but one fold per day were injected by non selective inhibitor NO- synthase-L-NAME (25 mg/kg during 42 days). Control was injected 0.95% NaCl. Animals were sacrificed after 24 hours after last drug administration. The nitrite (NOx) and free amino acids were measured in blood plasma. Free amino acids were determined by ion exchange chromatography.
Results: Chronic alcohol exposure resulted in increasing of histidine concentration in plasma and did not change the nitrite level. Treating with L-NAME has been shown to result in rather decreasing of NO concentration in plasma and histidine level normalization.
Conclusions: It is supposedly that chronic alcoholisation supressed the involving of histidine in histamine synthesis whereas NO level decreasing caused the normalization of histidine utilization. These findings may be evidence of substantial relationship between NO-synthase-system and the endogenouse histamine pool formation.

[18] P

Influence of amitriptyline on DAO and HNMT mRNA expression in different guinea pig tissues and on DAO release in guinea pig

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A part of proved anti-inflammatory action of antidepressants can arise from their effect on histamine elimination from the side of inflammation. Our studies have shown that antidepressants, amitriptyline (AMI) and sertraline change guinea pig histamine metabolism, affecting activity of diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT) in vitro. A single intraperitoneal dose of AMI also changes the profile of the heparin-induced release of DAO in guinea pig. The present study showed effects of two repeated doses of AMI on DAO and HNMT mRNA expression in different guinea pig tissues and on DAO release in guinea pig. Animals were treated with two doses of AMI (4 mg/kg, ip.) with time interval of 24 hours. Heparin (0.8 ml/kg iv.) was given 1 hour after the last dose followed by collecting blood samples to measure DAO activity in plasma using radiometric assay procedure. Additionally tissue samples were taken from the same animals and mRNA of DAO and HNMT was assessed by RT-PCR. The results showed that AMI inhibits immediate heparin-induced release of DAO into plasma. On the contrary, DAO activity in plasma rose slowly, showing dose-dependent effect. One hour after heparin injection, DAO activity in plasma was significantly higher in animals which received two doses of AMI in comparison to our previous results obtained after a single dose. This effect of AMI might be due to DAO release from tissues and also to changed expression profile of DAO mRNA. Our results showed that in AMI treated animals, DAO was expressed also in the tissues which normally do not express DAO or contain a small amount of DAO in untreated animals. In addition, HNMT induction was also noticed in AMI treated animals. It can be concluded that AMI diminishes rapid heparin-induced DAO liberation into plasma in vivo conditions and we demonstrated induction of DAO and HNMT mRNA expression which could be reason for gradual DAO activity augmentation in plasma.

[19] P

Histaminergic and orexinergic interference with clock gene controls hippocampal synaptic plasticity

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Histamine neurons in the posterior hypothalamus project widely through the whole brain to control a variety of brain functions, including behavioral state and memory processing. Mechanistic and molecular signatures linking histaminergic control of synaptic plasticity to memory functions have been delineated by pharmacological and electrophysiological means. Histamine via actions on H_1-, H_2-, H_3-, and NMDA-receptors modulates the release of excitatory and inhibitory transmitters, and affects intrinsic properties of hippocampal neurons in a spatiotemporally coordinated manner supporting synchronous network activity and synaptic plasticity. Using mice deficient in the clock gene PER1 we have begun to analyze the role of histamine and orexins in the entrainment of hippocampal molecular clockworks and bidirectional long-term synaptic plasticity, including synaptic tagging and capture (L-LTP/L-LTD). Results of these experiments will provide insight in molecular mechanisms controlling behavioral state-dependent learning and memory, which is relevant for a variety of disorders of feeding, mood, sleep, and memory.

[20] P

Toward regulation of food intake in portocavally shunted rats

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Portocaval anastomosis (PCA) induces chronic liver failure with resulting marked metabolic changes including these in brain histamine (HA) system. Among many functions HA system has regulatory role in feeding behaviour, HA acting as inhibitory modulator. However, PCA rats, despite their much higher brain HA content, eat and drink more than control rats do. Underlying regulatory mechanisms were therefore addressed. PCA and SHAM rats 6 mo after operations were used. Rats were rendered motivated for food intake by 16 hours fasting. Chlorphenmiramine (100 nmol) and ranitidine (200 nmol) were applied icv while SB 334867, an orexin (OX1) receptor antagonist (10 mg/kg) ip, 5 min and 30 min, respectively, before food presentation. The records were done hourly for 6 hr and at 24hr. The shunted rats, smaller by weight (321±34 vs 453± 17 g) ate and drank daily significantly more, i.e. 4.9±0.2 vs 3.3±0.3 g/100g bw and 11.1 ±2.4 vs 5.7±1.2 ml/100 g bw. They also excrete 2.5 fold more urine. More susceptible to orexin antagonist were the sham rats; a reduced food intake was present over 24 hr. Surprisingly, chlorpheniramine was a potent inhibitor of food intake in both groups, the sham rats again being more susceptible. We conclude: i/ the orexigenic system seems to compensate for activated HA activity in PCA rats, ii/ undesired chlorpheniramine effect may involve interaction with 5HT system; its activation in PCA rats may render them less sensitive to the drug. Studies are on way with another H_1 blocker, pyrilamine.

[21] P

Central serotonin-induced pressor effect in rats is mediated via the histaminergic system

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Histamine, acting centrally as a neurotransmitter, produces an increase in mean arterial pressure (MAP) and heart rate (HR) in anaesthetised animals. There are interactions between the histaminergic and other neuronal systems, including opioidergic, cholinergic, noradrenergic and angiotensinergic systems, in the central cardiovascular regulation. Our previous studies show that centrally acting serotonin re-uptake inhibitor citalopram also is able to affect central histamine-induced cardiovascular effects in rats. Therefore, present studies were undertaken to determine possible interactions between the histaminergic and serotonergic systems in the circulatory regulation. Experiments were performed on male Wistar-Kyoto rats under ketamine/xylazine anaesthesia. We measured MAP, HR and renal, mesenteric and hindquarters blood flow using the pressure transducer RMN-201 (Temed, Poland) and Transit Time Flowmeter type 700 (Hugo Sachs Elektronik, Germany), respectively. Renal (RVR), mesenteric (MVR) and hindqarters (HVR) vascular resistance were calculated by dividing MAP by peripheral blood flows. Serotonin (5-hydroxytryptamine creatinine sulfate complex, 5 nmol/5 μl) given intracerebroventricularly (icv) caused a rapid lasting 10-15 min increase in MAP and a decrease in HR, maximally up to 20% and 15% of the initial values, respectively. The effect was associated with increases in RVR, MVR and HVR. Pre-treatment with H_1 receptor antagonist chlorpheniramine (25 nmol/5 μl, icv) significantly inhibited serotonin-induced effects. On the other hand, neither H_2 receptor blocker ranitidine (50 nmol/5 μl, icv) nor H_3/H_4 receptor antagonist thioperamide (25 nmol/5 μl, icv) influenced cardiovascular changes evoked by serotonin. In conclusion, we demonstrate that central serotonin-induced cardiovascular effects in normotensive rats are, at least in part, mediated by the histaminergic system, and histamine H_1 receptors are involved.

[22] P

The synergistic effect of histamine and IL-6 on NGF secretion from cultured astrocytes is evoked by histamine stimulation of IL-6 secretion via PKC-MAPK signalling pathway

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Histamine is an important regulator of NGF synthesis and secretion. Besides its direct stimulation of NGF production, histamine acts also indirectly, via interactions with different cytokines. We showed in the past, that histamine exerts strong synergistic effect with IL-6 on NGF secretion from cultured astrocytes. In the present work we further investigated molecular mechanisms of histamine-IL-6 interaction in the regulation of NGF production. As experimental model, we used primary cultures of rat neonatal cortical astrocytes, prepared from the brain of Wistar rats. The cells were pre-incubated with either PKC (GF109203X, Gö6976) or MAPK (PD98059) inhibitors for 1 hour. After pre-incubation, the cells were treated with 1 μM histamine in the presence or absence of 10 ng/ml of IL-6. Secreted IL-6 and NGF were determined in the culture medium by IL-6 or NGF-ELISA accordingly. Our results show that histamine strongly enhances IL-6 secretion from cultured astrocytes after 24h of incubation. PKC inhibitors and MAPK inhibitor all inhibit the stimulatory effect of histamine. GF109203X, which is highly selective for conventional PKC isoform, shows the most potent effect, and completely inhibits IL-6 secretion induced by histamine. All selected inhibitors also diminish synergistic effect of histamine and IL-6 (10 ng/ml) on NGF secretion, which has been only observed after pre-treatment of the cells by histamine for 24 hours. We conclude that activation of PKC-MAPK signalling pathway plays a crucial role in synergistic effect of histamine and IL-6 on stimulation of NGF secretion. In this process the stimulation of IL-6 secretion by histamine is of importance.

[23] P

Histamine effect on the expression of AtoS-AtoC two component system in Escherichia coli

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In bacteria, the biosynthesis of polyamines is modulated at the level of transcription [1]. Upon acetoacetate-mediated induction, AtoS-AtoC two-component system acts through its direct effects on the atoDAEB operon transcription to regulate positively the biosynthesis of short-chain poly-(R)-3-hydroxybutyrate (cPHB) biosynthesis, a biopolymer with many physiological roles [2, 3]. AtoC is the response regulator of the AtoS-AtoC two-component system and it functions as the positive transcriptional regulator of the atoDAEB operon genes [4], encoding enzymes involved in short chain fatty acid metabolism. We report here that histamine contributed to cPHB biosynthesis enhancement by AtoS-AtoC in *E. coli* that overproduces both components of the AtoS-AtoC two-component system, while bacteria that overproduce either AtoS or AtoC alone did not display this phenotype. The roles of polyamines and histamine on the transcription of atoS and atoC genes as well as that of atoDAEB(ato) operon were studied. Polyamine-mediated induction was tested both in atoSC positive and negative E. coli backgrounds by using β-galactosidase reporter constructs carrying the appropriate promoters patoDAEB, patoS, patoC. Histamine was also tested for its effect on the activity of ornithine decarboxylase and on the growth of polyamine-deficient *E. coli* cells.

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[24] P

Role of polyamines in the expression of histidine decarboxylase and histamine synthesis during mouse mast cell differentiation

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Mast cells leave the bone marrow as immature precursors that complete their differentiation within peripheral tissues under the influence of the microenvironment. Differentiation and maturation include the synthesis and accumulation of a number of inflammatory mediators including histamine, several mast cell proteases (mainly tryptases and chymases) and cytokines. Working with several cell lines which exhibit some phenotypic features resembling those of the mast cells, we have previously shown how treatments able to increase the intracellular levels of histamine simultaneously reduce the intracellular levels of polyamines (small polycations absolutely necessary for cell survival) and we have characterized the mechanisms behind this observation [1-3]. To gain insight into the putative histamine and polyamine metabolisms interplay in mast cells, in this work we have used bone marrow derived mast cells to study the role of polyamines in the expression of histidine decarboxylase (HDC), the enzyme responsible for histamine biosynthesis. We have carried out amine quantification by HPLC, expression analysis of HDC by Northern and Western blot and HDC enzymic activity measurements. Our results show that polyamines alter the expression pattern of HDC and histamine production during mast cell differentiation. However, further experiments will be required to elucidate the underlying mechanisms.

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[25]P

Excitation of histaminergic neurons by thyrotro-pin-releasing hormone

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Thyrotropin-releasing hormone (TRH) originally isolated from hypothalamus got its name through the capacity to stimulate thyroid-stimulating hormone (TSH) release. It turned out later that TRH receptors and the TRH degrading enzyme (TRH-DE) are expressed and occurring not in the pituitary alone but also in many brain regions. Clinical and experimental reports demonstrated a role of TRH in the modulation of locomotion, cognition, mood and sleep. They suggested functional role of TRH as a neurotransmitter or neuromodulator at the central neurons. The tuberomamillary nucleus (TMN) plays a prominent role in sleep-wake regulation. Several sleep related peptides like galanin and the hypocretins affect histaminergic neurons. We studied now the effects of TRH on the electrophysiological activity of histaminergic neurons employing single unit extracellular recordings on acute slices of 4 week old Wistar rats. In the majority of the TMN neurons a significant increase (on avarage by 70%) in the spontaneous firing rate was observed followed by receptor desensitization possibly through receptor internalisation. Two different TRH receptors are expressed in the posterior hypothalamus, their distribution at the single cell level in TMN is under investigation. As TRH dramatically reduces sleeping time in rats and combats excessive sleepiness in canine models of narcolepsy, the present findings indicate a role for TMN neurons in TRH induced arousal.

[26] P

Effect of H_1 antihistamines in the model of mesenteric ischaemia/reperfusion

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Mesenteric ischaemia/reperfusion (I/R) initiates a series of events that result in mucosal injury and lead to the production of reactive oxygen species (ROS). Tissue ischaemia is accompanied by the release of histamine and free radicals are supposed to act as endogenous histamine releasers. The aim of our work was to study the possible protective effect of H_1 antihistamines in the rat model of I/R induced by occlusion of the superior mesenteric artery. The extent of intestinal damage caused by I/R was recorded, the activity of myeloperoxidase (MPO) was measured, evidence of increased free radical production was assessed by the chemiluminescence (CL) response of the ileal samples and of the whole blood, and the effect of antihistamines on the CL response was studied *in vivo* and *in vitro*. H_1 antihistamines dithiaden (DIT) and loratadine (LOR) at the dose of 10 mg kg-1 were administered twice i.p. For in vitro experiments the concentrations of 10, 50, and 100 μM/l were used.
In *in vivo* experiments, I/R induced a pronounced haemorrhagic intestinal injury. There was a significant reduction of the extent of injury by both DIT and LOR treatment. A slight MPO increase after 30 min reperfusion was also inhibited by both antihistamines. Polymorphonuclear leukocyte (PMNL) count in whole blood was significantly increased after reperfusion in control groups, with reduced ability of PMNLs to produce radicals and a weaker reaction to PMA stimulation. DIT was able to prevent the increase of PMNLs, LOR was ineffective.
In *in vitro* experiments, DIT in a concentration dependent way inhibited the spontaneous and even more pronouncedly stimulated CL. LOR in spontaneous CL was ineffective and exhibited dual action on PMA stimulated CL.
The results obtained showed a protective effect of both H_1 antihistamines on mesenteric I/R induced injury, involving different mechanism. The action of DIT appears to operate by interference with activated PMNLs.

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[27] P

In vitro inhibition of rat basophilic leukaemia mast cell (RBL-2H3) degranulation by novel indane compounds

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Mast cells are known to play a critical role in the development of immediate hypersensitivity reactions and numerous other inflammatory processes. The present study was undertaken to investigate the effect of two diastereoisomers, PH_5 and PH_2, of an indane compound on the in vitro degranulation of a rat basophilic leukaemia cell line (RBL-$2H_3$). Secretion from mast cells was assayed by quantification of the released histamine and β-hexosaminidase. Quercetin (final concentration 50 μM) was used as a positive control.
Our data indicated that PH5 (at a final concentration of 10 μM) inhibited histamine release by 25.0±7.7% ($p<0.001$, n=6), and β-hexosaminidase release by 34.25±5.0% ($p<0.001$, n=6). In contrast, PH_2 (also at 10 μM) was less effective, inhibiting histamine release by 19.0±5.8% and release of β-hexosaminidase by 10.0±4.0%; this inhibition was not statistically significant ($p>0.05$, n=6) from baseline. The inhibitory effect of PH_5 on degranulation was dose-dependent at concentrations from 300 nM to 50 μM, with a maximum inhibition of 67.43±4.5% obtained at 50 μM. In comparison, quercetin inhibited histamine release by 74.32±2.0% and β-hexosaminidase release by 65.8±6.8% ($p<0.001$, n=6). In all cases, the release of histamine was closely paralleled by the release of the enzyme; no statistical ($p>0.05$, n=6) difference could be observed in any of the groups.
These results indicate that PH_5 is the active diastereoisomer and might be an interesting candidate for further examination with regards to new anti-histaminic.

[28] P

Confirmation of biological effects of ultramolecular dilutions. Effects of ultramolecular concentrations of histamine, 4-methyl-histamine and of adrenaline on the activation of human basophils

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Background: To test, in parallel with histamine, ultramolecularconcentrations of 4-methyl-histamine and of adrenaline on of human basophils activation induced by fMLP and by anti-IgE. Methods: Human basophils obtained from blood anticoagulated with EDTA were pre-incubated 15\' with the different compounds at room temperature. For the study of histamine derivatives, basophils were stimulated by an optimal fMLP concentration and basophils activation was analysed by the anti-IgE FITC/CD203c PE protocol. Results were expressed as ratio of CD203c up-regulation. In the adrenaline experiments, basophils were stimulated by anti-IgE and results were expressed in percentage CD63 expression on basophils membrane. Histamine, 4-methyl-histamine and adrenaline were tested at concentrations in the range of 10^{-4}/10^{-6} M and in the range of 10^{-30}/10^{-36} theoretical molar (TMC) concentrations.
Results: Histamine and 4-methyl-histamine induced a significant inhibition of fMLP induced basophils activation. Histamine dilutions 10^{-30}, 10^{-32} and 10^{-34} TMC were tested in parallel with the same concentration ranges of 4-methyl-histamine, inhibition being similary significant for 10^{-32} and 10^{-34} TMC. Adrenaline produced a significant inhibition of anti-IgE induced basophils activation, both at 10^{-4} M and in the range of 10^{-30} to 10^{-36} TMC. The inhibition was reversed by propanolol 10^{-6} M. Conclusions: In addition to the confirmation of the biological activity of high dilutions of histamine on the basophil activation model, these experiments show that ultramolecular concentrations of an H_2-receptor agonist (4-methyl-histamine) and of adrenaline mimick the inhibitory effects on human basophils activation induced by conventional concentrations of 4-methyl-histamine and of adrenaline, possibly acting at H_2 and ß receptors.

[29] P

Histamine augments beta2-adrenoceptor-induced cyclic AMP accumulation in human prostate cancer cells DU-145 independently of known histamine receptors

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Androgen-independent prostate cancer cells DU-145 express a number of G protein-coupled receptors, including histamine H_1 receptors. Since there is evidence for the presence of beta-adrenoceptors in the human prostate, in this work we set out to characterise the expression of beta-adrenoceptors by DU-145 cells, their linking to cyclic AMP (cAMP) formation and the possible modulation by histamine H_1 receptors of beta-adrenoceptor function. Saturation [3H]-dihydroalprenolol binding indicated that DU-145 cells express moderate levels of beta-adrenoceptors (22.7 +/- 2.5 fmol/mg protein, Kd 0.37 +/- 0.03 nM), which belong to the beta2-subtype as assessed by antagonist inhibition (ICI-118,551 and CGP-20712A). In [^{3}H]-adenine-labelled cells, beta-adrenoceptor stimulation with isoproterenol resulted in robust [^{3}H]-cAMP accumulation (10- to 30-fold of basal, EC50 142 nM; pEC50 6.85 +/- 0.05). While not having effect of its own on basal [^{3}H]-cAMP accumulation, histamine significantly augmented the beta2-adrenoceptor-induced response (overall effect 152 +/- 6% of isoproterenol alone) in a concentration-dependent manner (EC50 1.35 micromolar, pEC50 5.87 +/- 0.06). Histamine enhanced the maximum response to isoproterenol, but had no significant effect on either the agonist EC50 (pEC50 6.92 +/- 0.11 versus 6.89 +/- 0.10 for controls) or Hill coefficient (nH, 0.90 +/- 0.16 versus 0.84 +/- 0.13). The histamine enhancing action was independent of extracellular Ca^{2+}, insensitive to antagonists/agonists at H_1, H_2 or H_3/H_4 receptors and mimicked by drugs containing an imidazole ring in their chemical structure and by imidazole itself. Taken together, our results show that in DU-145 cells histamine augments beta2-adrenoceptor-induced cAMP independently of the activation of known histamine receptors. The effect may involve other mechanisms such as allosteric modulation of $beta_2$-adrenoceptors by the imidazole moiety of histamine.

[30] P

Epigallocatechin-3-gallate inhibits key features of mast cells related with inflammatory responses

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Mast cells are key cellular mediators of inflammatory responses. Epigallocatechin-3-gallate (EGCG) is a natural bioactive compound present in green tea, which has been previously shown to have potent antioxidant, antiinflammatory and antiangiogenic effects. The goal of the present work was to study the modulatory effects of EGCG on human mast cell expression of a panel of angiogenesis-related genes, by using a functional genomics approach. Our results show that EGCG induces partial inhibiton of the expression of two integrins and monocyte chemoattractant protein-1 (MCP-1), as observed in macroarrays and confirmed by RT-PCR. Furthermore, these effects on gene expression are functionally validated by showing inhibitory effects of EGCG on key features of mast cells, as shown in adhesion, aggregation, migration and recruitment assays.
In conclusion, our results add new biological targets of EGCG contributing to explain its pharmacological effects as an antiinflammatory and antiangiogenic compound.

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[31] P

H_4 receptor mediated effects of histamine on mouse dendritic cells

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Dendritic cells (DCs) are the main antigen presenting cells and play a crucial role in the initiation of the immune response. The ability of DCs to coordinate the immune system is an intrinsic quality of these cells, but the actual direction of the arising immune response is rather a result of the environmental stimuli, which are reflected in the cytokine and chemokine production of the DCs. Histamine, as an inflammatory mediator, is known to affect DC functions. Beside histamine H_1 and H_2 receptors, DCs are known to express the newly discovered histamine H_4 receptor (H_4R) but its function on the surface of DCs has not been completely elucidated. We demonstrated previously, that H_4R-/- mice have reduced number of CD8+, CD11c+, lymphoid DCs. In recent studies we found decreased migratory capacity of H4R-/- DCs, which is in accordance with our findings that H_4R-/- DCs express much lower CCR7 than their wild type counterparts. On the other hand in vitro antigen presentation was elevated in H_4R-/- DCs. The results of our experiments performed with specific H4R antagonist JNJ7777120 confirmed that H_4R signaling is involved both in DC migration and antigen presentation. Lack of H4R had no influence on DC cytokine expression however selective H_4R antagonist seemed to compensate the suppressive effect of histamine on Th1 cytokine INFγ but not IL-10 production.
These results indicate that H_4R activation is an important signal for DCs as it affects maturation, cytokine production as well as migration of these cells. These observations suggest new potential applications for the novel H_4R ligands in vaccination therapy of cancer.

[32] P

The lack of H_4 receptor has a significant impact on the T cell development of H_4R-KO mice

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Hemopoiesis is regulated by different factors, including low molecular weight substances, like histamine. The effect of histamine is markedly dependent on the types of histamine receptors, expressed on target cells. Hemopoietic cells express H_1-, H_2 and H_4 receptors.
The aim of this study was to characterize the T cell development in the thymus of H4R-KO and wild type mice.
We used multicolor FACS stainings for the detection of T cell precursors and the different cell types involved in thymic negative and positive selection processes. In order to investigate the intrathymic migration processes, the gene expression profile of CD4+/CD8+ double positive thymocytes was studied by microarray analysis. While the percentages of the lymphoid stem cells were similar in the thymus of the investigated groups, significantly higher percentages of pro T cells and elevated frequency of late pre T cells could be detected in H_4R-KO animals. In the generation of a functional T cell repertoire thymocytes, expressing nonfunctional or autoreactive TCR, are deleted by apoptosis. In H_4R-KO mice the percentage of thymic CD3dim+/CD95L+ cells was elevated. In thymus apoptosis is induced by interactions that involve TCR on thymocytes and self-peptide-MHC complexes on thymic stroma cells. The expression levels of MHC-II molecules was significantly lower in H_4R-KO mice compaired to the wild type group. On the other hand, the percentage of CD11chigh+ dendritic cells was decreased significantly in H_4R free animals. IL-7 has a crucial survival role during CD4-/CD8- thymocyte development. A significantly lower percentage of IL7R+ cells and IL7R+ lymphoid stem cells were found in the thymus of H_4R-KO animals.
Our results suggest that the lack of the effect of histamine through H_4 receptor has a general impact on T cell development.

[33] P

Novel potent dual histamine H_1/H_3 receptor antagonists

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The classical concept of one drug-one target is not necessarily true for many therapeutic approaches, e.g. histamine H_1 receptor antagonists widely used for treatment of allergy nasal disease do not meet all aspects of the illness. Few studies have investigated the involvement of histamine H_3 receptors in nasal allergy. In animal nasal studies the combined administration of an histamine H_1 and H_3 receptor antagonist in low doses have led to synergistically inhibitory efficacy in comparison to single drug administration, where even no effect could be observed at the same dosage range [1]. Therefore, dual antagonist of both receptors, in which an histamine H_1 antagonist pharmacophore is linked to or incorporated into an histamine H_3 antagonist pharmacophore, might offer advantages for treating upper airway allergic responses [2]. This hybrid approach was realized by combination of 1-(3-(phenoxy)propyl)piperidine structure as a non-imidazole histamine H_3 receptor antagonist with a desloratadine structural element for hH_1 receptor binding via aliphatic spacers of different lengths. H_1 receptor binding was performed using [^{3}H]mepyramine on CHO-K1 cell lines transfected stably expressing the hH1 receptor. Data of histamine H_3 affinity was obtained by [125I]iodoproxyfan binding on CHO-K1 cells stably expressing the hH_3 receptor [3]. In spite of coupling with an histamine H_1 receptor antagonist pharmacophore the novel histamine H_3 receptor antagonist showed subnanomolar affinities for histamine H_3 receptor and nanomolar affinities for histamine H_1 receptor. The results demonstrate the possibility of non-imidazole hybrid structures with a dual histamine H_1-H_3 antagonist profile.

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[2] A. Hüls et al., Bioorg. Med. Chem. Lett. 1996, 6, 2013-18.
[3] M. Garbarg et al., J. Pharmacol. Exp. Ther. 1992, 263, 304-10.

[34] P

Comparison of piperidine vs. piperazine derivatives as histamine H_3 receptor antagonists

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The histamine H_3 receptor is mainly presynaptically located, constitutively active Gi-protein coupled auto-and heteroreceptor, mostly expressed in central nervous system (CNS). Blockade of this receptor leads to increased levels of histamine and other neurotransmitters such as: ACh, NA, 5-HT in CNS. Therefore histamine H_3 receptor antagonists may provide novel therapies for treating such CNS diseases as: Alzheimer disease, ADHD, dementia, Parkinson's disease, epilepsy, obesity [1]. Based on our previous research and on recent results described in literature [2], we synthesized the series of compounds in the group of ether derivatives of 3-piperidinopropan-1-ol. We obtained also corresponding and related piperazine derivatives in which one nitrogen basicity has been varied or decreased by alkylation arylation or acylation in 4-position. The novel compounds were evaluated for histamine H_3 receptor activity in vitro in a binding assay for the histamine H_3 receptors stably expressed in CHO-K1 cells on [125I]iodoproxyfan displacement. Compounds presented show no to relative good H_3 receptor affinities. Comparing. the histamine H_3 receptor affinities of the two structural classes of related derivatives made it obvious that most of the corresponding piperazine derivatives possess no detectable H_3 receptor affinity, even if the basicity of the nitrogen in 4-position was decreased. However, two of the compounds in this group showed similar, or slightly lower histamine H_3 receptor affinities compared to that of the corresponding piperidine derivatives
The results show that the replacement of piperidine with 4-N-substituted piperazine moieties does not always result in a total loss of histamine H_3 receptor affinity (e.g. at least two of the tested compounds), but that the possibilities for structural variations are strongly restricted.

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[35] P

Antiinflammatory effects of sulfated heparin-like semi-synthetic derivatives in carrageenan-induced model of inflammation in rat

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The K5 polysaccharide obtained from *Escherichia* coli strain 010:K5:H4 is a polymer of the disaccharidic unit formed by D-glucuronic acid and N-acetylglucosamine. This structure is akin to N-acetylheparosan, the precursory polymer of heparin and of heparin sulphate. This structural affinity with N-acetylated heparin with the sulphated heparin makes the K_5 polysaccharide extremely useful for the preparation of sulphated heparin-like semi-synthetic derivatives. Heparin has several biological actions independent of its well-known anticoagulant activity, including the ability to modulate extracellular matrix synthesis, cellular proliferation, angiogenesis, and inflammation (1,2). The aim of the present study was to evaluate the effects of a heparin-like semi synthetic derivative (K5 NS OS epi 0.1-0.5-1 mg/kg b.w.) on a well-known *in vivo* model of inflammation, such as carrageenan-induced pleurisy in the rat. Injection of 0.2 ml of 1% λ-carrageenan into the pleural cavity elicited an acute inflammatory response characterized by fluid accumulation in the pleural cavity which contained a large number of polymorphonuclear neutrophils (PMNs) as well as an infiltration of PMNs in lung tissues and subsequent lipid peroxidation, as documented by the increase in MPO activity and MDA production, and increased production of PGE_2, TNF_α and IL-1β. Furthermore, carrageenan induced an activation of lung mast cells and an upregulation of COX-2 protein expression. All the parameters of inflammation were dose-dependently and significantly reduced by the K5 NS OS epi compound and by celecoxib, 1mg/kg b.w, used as positive control. The compound B4/110, the reference molecule not endowed with antiinflammatory activity was completely inactive. These results indicate that sulphated heparin-like semi-synthetic derivatives and in particular N-sulphated and epimerized K5 derivative is able to inhibit both the expression and the production inflammatory mediators and offers a new therapeutic approach for the management of various inflammatory diseases.

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[36] P

Gastric effects of the highly selective histamine H_3 receptor agonist methimepip

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Previous data [1] showed that HCl-induced gastric lesions were reduced by R-(α)-methylhistamine but not by the histamine H_3 receptor agonists immepip and imetit. Moreover, it was recently observed that H_4 receptor antagonists mediate protective effects against gastric lesions induced in the rat by indomethacin [2]. Since both immepip and imetit behave as mixed H_3/H_4 receptor agonists, in the present study we investigated the effects of the highly selective H_3 receptor agonist methimepip [3] and immethridine, in comparison with immepip, against the gastric lesions induced by HCl or indomethacin. Methimepip (30 and 100 mg/kg intragastrically, ig) did not modify gastric lesions induced by indomethacin 20 mg/kg subcutaneously, sc, or by 0.6 N HCl ig. However, this compound, administered sc at 30 mg/kg, induced a significant inhibition of HCl-induced lesions; by contrast methimepip (30 mg/kg sc) did not modify indomethacin-induced damage. Immepip (30 and 100 mg/kg, either ig or sc) was inactive against gastric lesions induced by indomethacin or by HCl. Immethridine (30 mg/kg sc) significantly reduced HCl-induced lesions (approximately 62% inhibition) and this effect was prevented by the selective H_3 receptor antagonist UCL-2138 (4) (10 mg/kg sc). In conclusion, the use of selective H_3 receptor ligands confirmed that the activation of H_3 receptors mediate protective effects against gastric damage induced by necrotizing agents. Pharmacokinetics of H_3 receptor agonists and/or their affinity at H_4 receptors may explain the discrepancies observed across the experimental assays.

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[37] P

Antiinflammatory, analgesic and gastric effects of the novel and selective histamine H_4-receptor antagonists VUF10214 and VUF10148

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Previous data have shown that the selective histamine H4 receptor antagonists JNJ7777120 and VUF6002 induced in the rat antiinflammatory and analgesic effects combined with protection of the gastric mucosa [1]. The present study examined the antiinflammatory, analgesic and gastric effects of the novel histamine H4 receptor antagonists, VUF10214 and VUF10148 in comparison with VUF10181, an inactive analog of VUF10148. In the same experimental models the non selective cyclooxygenase inhibitor indomethacin (IND) was used. Inflammation was induced in rats by subplantar injection of carrageenan (CARR) into the left hind paw. Paw edema and thermal hyperalgesia were measured immediately before CARR injection and thereafter at 2, 4 and 6 hrs. The development of gastric lesions was assessed 6 hrs after administration of the compounds. "Lesion index" was determined by measuring each haemorrhagic lesion along its greatest length (mm).
Both VUF10214 (10 mg/kg, subcutaneously, sc) and VUF10148 (30 mg/kg, sc), significantly inhibited either paw edema (maximal reduction approximately 53%) or thermal hyperalgesia (maximal increase of paw withdrawal latency approximately 70%) induced by CARR. By contrast, the inactive analogue of VUF10148, compound VUF10181 (30 mg/kg, sc) was ineffective in paw edema assay. Neither VUF10214 nor VUF10148 induced damage to the stomach at any dose tested (10-30 mg/kg, sc), whereas IND provoked gross hemorrhagic lesions in the gastric mucosa (lesion index: 23.50±4.91 mm).
In conclusion, our study showed that the histamine H4-receptor antagonists VUF10214 and VUF10148 had antiinflammatory and analgesic activities, which were not associated with gastric damage. In line with previous data [1] these findings confirm that H4 receptor antagonists may represent new gastrosparing anti-inflammatory drugs.

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[38]P

Coupling mRNA expression to functional assessment of histamine receptor activity in the avian intestine

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Histamine is a potent stimulant of intestinal anaphylaxis, inducing epithelial ion transport and smooth muscle contraction in avian species. In mammals, histamine acts on cells of the intestine including smooth muscle, epithelial and neuronal cells via multiple pharmacologically distinct G-protein coupled receptors. The mechanism of action of histamine is poorly defined in the chicken intestine, however, and the subtypes of receptors through which histamine acts are unknown. The purpose of this study, therefore, was to demonstrate expression of histamine receptor mRNA and characterise receptor involvement in histamine-mediated smooth muscle contraction and epithelial ion transport.
RT-PCR confirmed the presence of three histamine receptor subtypes $H_{1/2/3}$, expressed at an mRNA level in both mucosa and smooth muscle. Using classical pharmacological techniques, we demonstrated that smooth muscle contraction occurred exclusively via H_1 receptor activation (EC_{50} 3μM, N=38) and was insensitive antagonism of $H_{2/3/4}$ receptors or to neuronal blockade using tetrodotoxin (1μM; N=5). In contrast, histamine stimulated a biphasic ion transport response, producing an effect at low concentrations (EC50 10 nM; N=45) which was sensitive to H_1 and H_2 receptor antagonism using mepyramine (1μM) and cimetidine (1μM) respectively and tetrodotoxin-insensitive as well as a distinct effect at high concentrations (EC_{50} 3μM; N=45) which was abolished by $H_{3/4}$ receptor antagonist thioperamide and tetrodotoxin-sensitive (1μM). Furthermore, while the H_2 receptor agonist dimaprit and $H_{3/4}$ receptor agonist r(α)methylhistamine had no significant effect on basal smooth muscle tone, both stimulated increased epithelial ion transport (N=6). In conclusion, while both regions of the chicken ileum express mRNA for multiple histamine receptors, functional studies confirm a more complex role for histamine in this tissue.

[39] O

Keyword-based, Boolean-logic driven data mining discloses correlations between enigmatic idiopathic conditions, occlusive vascular diseases such as tropical endomyocardial fibrosis (EMF) with unexplained eosinophilia, and "histamine dysmetabolism"

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Clarifying disease mechanisms in which there are concurrent risks to toxic factors and to protein-energy malnutrition (the single greatest health hazard to children) is critically important. To this end, linkage of logically related noninteractive evidence better enables health organizations to implement preventative countermeasures. The etiology of tropical EMF is little-known. The basic lesion, first reported half a century ago, is obliterative ventricular endocardial fibrosis. A World Health Organization Expert Committee estimated that such eosinophilic heart disease causes 10-20% of all cardiac deaths in tropical Africa and Southeast Asia. Tropical EMF primarily affects children and young adults and peri-operative mortalities approach 20%. Protein-poor cassava is a staple for about a half-billion people and consumption of uncooked cassava induced EMF in experimental monkeys.
Cassava cyanogensis cell rupture induces release of cyanide (CN-) from endogenous antifeedants. About 35 mg HCN (about half a lethal dose) could be incurred by an adult who consumes 750 g day 1 of insufficiently processed cassava. One detoxification mechanism in animals for CN- is sulfuration to form thiocyanate (SCN-). In humans, about 90% of SCN- filtered by the kidneys is reabsorbed. A common side-effect of SCN- treatments (introduced in the 1930s for hypertension) was iatrogenic blood eosinophilia. SCN- is a major substrate for eosinophil peroxidase. CN- & SCN- are potent inhibitors / ligands of several enzymes including some that degrade histamine. Circulating eosinophils also are "natural antihistaminics".

[40] P

Histamine in red wine: no correlation between histamine level and wine quality

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Histamine is claimed to be responsible for intolerance reactions such as flush, rhinorrhea, headache, and diarrhea occurring in some subjects after drinking red wine. As high levels of biogenic amines in wine may indicate poorly-controlled winemaking, particularly regarding malolactic fermentation, red wines of high quality may be expected to have low histamine levels and thus should be tolerated better by sensitive subjects. We investigated whether there is a correlation in red wines between histamine levels and corresponding scoring results from wine tasting. One-hundred Austrian red wines from different grape varieties grown locally or world-wide (vintages 2003-2005) were judged by a professional wine taster according to Parker's 100-point scale and subsequently analysed for histamine by immunoassay (Immunotech, Marseille, France). There was considerable variation in histamine content between wines (mean 8.4±7.5 mg/l, median 5.9, range 0.45-27.6) with no significant differences between the seven varieties investigated (Zweigelt, Blaufränkisch, St. Laurent, Pinot noir, Syrah, Cabernet Sauvignon, Merlot). No significant correlation was found between individual histamine values and wine rating scores in the whole data set (r=0.13) as well as within single varieties. Although wines high in histamine on average had better wine scores, each score class contained wines with very high as well as with very low histamine levels. Different wines from the same winemaker usually had similar histamine content indicating a critical influence of company-specific factors during the winemaking process on the generation of histamine. The data show that red wines may show considerable variation in histamine levels independent of grape variety. As there does not seem to exist a clear relationship between sensoric wine quality and histamine content, histamine-sensitive subjects are unlikely to develop less symptoms from high-rated wines.

[41] P

Substance P-induced cyclooxygenase-2 expression in polymorphonuclear cells

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Substance P (SP) is a neuropeptide involved in neurogenic inflammation and able to induce prostaglandin (PG) production in various cell types. We have shown that SP evokes COX-2 expression in human umbilical vein endothelial cells by interacting with NK_1 and NK_2 receptors. Polymorphonuclear cells (PMNs) produce eicosanoids and are responsive to SP as demonstrated by our group. Experiments were performed to evaluate whether or not SP induces COX-2 expression in human PMNs, isolated from the peripheral blood of healthy volunteers, that was obtained according to the italian law. Protein expression was analysed by Western Blot; densitometric analysis was performed by Gel-Pro ®Analyser 4.5, 2000. COX-2 protein expression was upregulated by SP with a peak at 1nM (1pM-1μM) and at 5h (0-7h); in the same conditions COX-1 protein expression was unchanged. NK_1, NK_2 and NK_3 receptors on PMNs were detected by immunoblotting. Selective receptor agonists, namely [Sar9, Met(O2)11]SP, -Ala8] NKA(4-10) and senktide, evoked a response of the same order as that following an equimolar concentration of SP. L703,606 and SR48,968, selective NK_1 and NK_2 antagonists, respectively, reverted SP-mediated effect on COX-2 expression, being SR48,968 more active. The signalling pathway underlying SP-induced COX-2 expression included MAPKs. A time-dependent MAPKs p38, p42/44 and p54 JNK activation, but not p46 JNK, was measured. SP-induced COX-2 expression was inhibited by selective inhibitors of the three kinases. SP induced NF-kB activation and even the NK selective agonists evoked p65 nuclear translocation, but at a less extent than SP. Dexamethasone (0,1 μM) inhibited SP-mediated COX-2 expression, thus suggesting an effect at the nuclear level. The study shows human PMNs to possess NK_1, NK_2 and NK_3 receptors and suggest that all the three receptors contribute to mediate COX-2 expression by SP in human PMNs.

[42] P

Effect of histamine chloramine on luminol-dependent chemiluminescence of granulocytes

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At a site of inflammation activated granulocytes generate large amount 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). 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 ($HisCl_2$). We found that HisCl inhibited luminol-dependent chemiluminescence (CL) of granulocytes in a dose-dependent manner (10^{-6}-10^{-3} M). Thioperamide H_3/H_4 reverse agonist completely bloked the inhibitory effect of HisCl on CL. Pyrilamine and cimetidine at all concentration tested (10^{-6}-10^{-4}M) were unable to antagonize the inhibitory effect of HisCl on oxydative burst of granulocytes. 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.

[43] P

In vitro-tests with human blood cells are a practicatool in the immunological risk assessment of chemicals

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In recent research an allergen list was developed for sensitizing risk assessment in the REACH (Registration Evaluation Authorisation of Chemicals) product registration [1]. The immunological examination of chemicals and products is demanded. In vitro-tests like the Histamine Liberation Test (HLT) in combination with the Basophil Degranulation Test (BDT) and the Lymphocyte Stimulation Test (LST) are recommended to minimize animal experiments. Silicate based interior paint was used for the improvement of the validity of HLT, BDT and LST. A commercially available silicate based interior paint peripheral blood was taken from an adult atopic (IgE > 1000 IU) and from a sex and age matched non-atopic (IgE < 100 IU) control. The basophil-containing PBMC were separated by gradient density centrifugation. 3 x 10^7 cells/ml were pre-incubated at 37°C for 15 min. 2 x 10^{-5} and 2 x 10^{-3} g/l of the silicate based interior paint were added. After 30 min histamine was measured using the HPLC/flourimetric technique [2]. FMLP and anti-IgE served as positive controls. The LST and cytokine measurements were performed as it was previously described [3]. Using the HLT no histamine liberation was detected even at the higher paint concentration. The sensitized basophil granulocytes did not show any increased degranulation (BDT). The results indicated that there is no distinct risk for the immediate allergic reaction after paint challenge. Surprisingly, a dramatic increase of the Th_2/Th_1-balance (allergotoxicological index = 8.3 [2,3]) could be observed in the LST. Therefore, a special sensitization effect must be considered in atopic patients, when they come into chronic contact with the examined paint. Due to the presented results it can be suggested that the HLT combined with the BDT and LST are practical human in vitro/ex vivo-methods for the assessment of allergenic/sensitizing potency of products and a good approach for the minimizing of animal experiments in REACH.

[1] AVE-Commission. (2006) Umwelt&Gesundheit 2, 47-53.
[2] Diel et al. (1998) Allergy 53, 1052-1059.
[3] Diel et al. (1999) Tox Letters 107, 65-74.

[44] P

Music and Histamine

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Music is an art and science that has been used since ancient times to influence various aspects of human life, including physical and spiritual, individual and social emotion. Music can execute its power on the entire personality, modifying the status of emotion, behaviour, including physiology [1]. Music provides a sound stimulus by the auditory apparatus that impresses the ear drum, which afterwards is processed by the limbic system and the hypothalamus. The peripheral glands are triggered, generating an internal response to neuro-endo-immunological targets. The question arises as to whether mechanisms of stimulus transduction can be reflected by histamine reactions. A group of volunteers (n=12, age 19-41) was selected from a university student course (no further randomization). Routine anamneses including a special "music anamnesis" (MA) revealed the individual type of "good feeling music" (GFM) [1]. Histamine was determined after venipuncture and ca. 30 min pre-injection with anticoagulant. The students were exposed to GFM at least 3 times for 5 min. Just after music exposure blood was drawn again for histamine measurements [2]. Plasma average histamine (0.55 ± 0.3 ng/ml SEM) was suppressed (< 0.4 ng/ml = detection limit, $p = 0.1$ student t) after listening to GFM. No changes of histamine concentration could be measured after "regular" music and/or time of silence as it was defined in the personal MA. Furthermore, MA could predict e.g. the specific mode of asthma treatment:

-Rhythm can mimic the heart beat in harmony with the biological pulse including the respiratory system.
-The melody influences emotions, sensitivity and central responses to the respiratory system.
-The harmony provides for the physiological balance, supporting the mental ability for the control of adverse situations.

It can be concluded that beside routine clinical treatment music can influence the realisation of health care in particular the prevention of asthmatic diseases. The specific role of histamine in music therapy is still a tool of investigation in the future.

[1] Gigante Pérez C, Diel F. (2006) Sensus 15, 24-27.
[2] Horr et al. (2006) Int Immunopharmacol 6, 1577-1585.

[45] P

Histamine intolerance - diagnosis and therapy

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About 3.6 % Germans suffer from food hypersensitivity, 2.5 % IgE mediated [1]. Non-allergic reactions to food are often defined to be induced by biogenic amines, corresponding to symptoms like headache, rhinitis, respiratory, digestive complaints and eczema. Histamine intolerance (HIT) is almost not genetically induced but acquired by an infect of the intestinal mucosa, or reduced Diaminooxidase (DAO)-activity induced by medication, or by consumption of large quanitites of food rich in histamine and other biogenic amines.
In a retrospective study, 126 patients of the AVE were interviewed using an allergy and nutrition questionnaire [2]. Diagnosis: Oral provocation, and determination of histamine, DAO, and Vit B6 blood level when HIT was suspected. It could be shown that food can respond as a trigger of adverse reactions and histamine was produced by microbial spoilage or during intended processing of food of primary animal origin in spoilt fish (esp. tuna and mackerel) (35 %); salami and raw ham (24 %); long ripened cheese (23 %) and alcoholic beverages like red wine and beer (23 %).
Histamine releasing food was indicated e.g. strawberries, citrus fruits, tomatoes, seafood, and alcohol as typical histamine liberators. Furthermore, drugs (e.g. ACC, Ambroxol, Theophyllin) were identified as inhibitors of DAO activity. This may induce an increased local contamination of histamine in the organism. Additionally some antirheumatics and analgetics were also identified as typical histamine liberators. In the present study it was suggested that amine-poor diet, medication (H_1-receptor blocker) and food supplements (Vitamine B6 and C, DAO, buffering salts) are needed. Furthermore, food industry should be requested to lower the histamine content by selective control of manufacture and use of histamine-poor technologies. Poor histamine content provides the criteria for food quality check [3].

[1] Zuberbier et al. Allergy. 2004;59: 338-45.
[2] Diel and Diel, Allergien , Moewig (Rastatt 1997)181-97.
[3] Bodmer et al. Inflamm Res 1999;48 6:296-300.

[46] P

Symptoms in double blind placebo controlled histamine provocation

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Patients with histamine intolerance (HI) suffer from H mediated symptoms after the ingestion of histamine rich food. The aim of this study was to establish a reliable method for the diagnosis of H intolerance respecting the symptoms in combination with plasma H levels. A rise of at least 40% of the plasma H level before provocation combined with at least 1 symptom after provocation was the definition for the diagnosis of HI. 18 persons were tested with a drink of histamine of placebo (in peppermint tea). In an interval of 10 min patients were asked for symptoms for 1 hour. Blood pressure and heart frequency was taken continuously, and to measure respiratory symptoms the peak expiratory flow was taken regularly. After 1 hour they were asked to document the symptoms themselves. At the same time blood was taken to measure H levels. There were many subjectively experienced symptoms reported such as headache, vertigo, stomach ache, heat sensation, tiredness. Besides these symptoms there was diarrhea, swelling of nasal mucosa, flush and meteorism documented. There were 4 patients fitting the diagnostic criteria of HI. 10 patients had or were suspected to have gastrointestinally mediated allergy. 78,6% of the patients with HIT or GMA were symptomatic after H provocation, while only 26,6% of this patient group had a rise of plasma H level. Vice versa the 4 control persons did not refer any symptoms, but all of them had a rise of plasma H level. After placebo provocation 54,5% of the patients referred symptoms, while 18,2% had a rise of plasma H level. There is no correlation between rise of plasma H level and symptoms, even after placebo provocation over 50% of the patients referred symptoms. There might be a psychosomatic influence. Another possibility is that the peppermint infusion used in verum and placebo group can cause symptoms in patients with salicylate intolerance. So in the following tests NaCl solution alone should be used to dissolve the histamine.

[47] P

Plasma histamine levels in double blind placebo controlled histamine provocation

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Histamine intolerance (HI) is known as misbalance between histamine (H) present in the body and H degrading enzymes. Patients with HI show H mediated symptoms after ingestion of H rich food such as mature cheese, red vine, tuna, tomatoes. The aim of this double blind placebo controlled H provocation was to establish a good diagnostic tool for the diagnosis of HI. Eighteen persons were tested at intensive care unit under continuous surveillance of vital parameters. Each person was tested twice. Verum, consisted of 75 mg H mixed with 250 ml peppermint infusion and 250 ml 0,9% NaCl fluid, placebo consisted just in peppermint infusion and NaCl fluid. In time intervals of 10 min blood was taken to measure plasma H levels for 1 hour. The definition for the diagnosis was a rise of plasma H levels of at least 40 % of the H level before provocation and any symptoms beginning after the intake of the provocation fluid. There were 4 patients who fulfilled the diagnostic criteria for HI. 4 persons were tested as control group (CG), 10 patients had the diagnosis or the suspect of a gastrointestinal mediated allergy (GMA). In the group of patients with HI there was no significant difference between plasma H levels under placebo or verum. Placebo H levels were stable (0,23+-0,11; 0,28 +-0,18), H levels after H provaction ranged between 0,20 +- 0,11 and 0,30 +-0,29 with a slightly rising dynamic. Control group reached the highest H levels (0,42 +-0,07) after H provocation. There was no significant difference of the H levels between the 4 groups neither after H provocation nor after placebo provocation. Therefore for the following tests a higher dosage of 150 mg H should be applied. The observation that control persons did not feel any symptoms with the same plasma histamine levels or even higher levels and the same rising dynamic of histamine levels shows that there must be still another trigger in patients with histamine intolerance symptoms, i.e. a more sensitive H receptor.

[48] P

Evaluation of ECP release of intact tissue biopsies from patients with nasal polyps

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The stimulation of functional intact tissue under in-vitro conditions with the biopsy mucosa oxygenator has been previously established in the diagnostics of gastrointestinal diseases. The aim of this investigation was to assess the applicability of this system also for nasal tissue. With the eosinophilic cationic protein (ECP) released by activated eosinophils a keystone marker for inflammatory processes in the nose was used.
Immediately after sample taking during endonasal sinus surgery 30 biopsies from nasal polyps in total were incubated in an oxygenated, physiologic culture medium for 300 minutes (biopsy mucosa oxygenator; INTESTINO-DIAGNOSTICS, Erlangen). All biopsies were taken from the ethmoid sinus from polypoid tissue and from the lower turbinate reflecting normal tissue (control). Eosinophilic cationic protein was measured by ELISA (Beckmann-Coulter, Krefeld) and its release (median, range) was assessed as kinetics over time and is given as net release (ng/mg w.w.).
The excretion of ECP from normal turbinate tissue was similar in all individuals over time (net release 0.23 ± 0.12). Hereby it is documented that the system used here is able to keep the vitality of nasal tissue. The kinetics showed that biopsies from nasal polyps had increasing rates of ECP release (net release 2.14 ± 1.9) with already 8 to 12 fold increase from the beginning compared to controls
These data confirm that biopsies from patients with nasal polyps show increasing rates of ECP due to activated eosinophils within polyp tissue. This method is also applicable for further research on sinus disease, especially directed towards nasal polyps and aspirin-exacerbated respiratory disease (AERD).

[49] P

Reduced asthma symptoms and altered gene expression profile in histamine deficient mice

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Histamine is an important mediator released from activated mast cells provoked by allergen and has a substantial role in the pathophysiology of asthma. Several lines of evidence indicate, however, that histamine could also have important functions in the regulation of basic cell biological processes. We have used histidine decarboxylase gene-targeted (HDC KO) mice, lacking histamine, to investigate the effect of histamine deficiency on the development of asthma. Our previous investigations revealed that HDC KO mice had fewer mast cells with reduced granular content and defective degranulation characteristics. Ovalbumin (OVA) sensitized and challenged HDC KO mice had significantly reduced airway hyperresponsiveness, lung inflammation, BAL eosinophilia, and OVA-specific IgE compared with congenic wild type (WT) littermates treated in the same way. Comparing the whole lung gene-expression profiles of the different groups in more than 20.000 genes revealed significant differences in the HDC KO mice in asthmatic late phase, indicating a significantly altered immune response to OVA provocation and challenge. Evaluation of the cytokine and chemokine gene expression revealed that OVA-treatment caused elevation of both Th1 and Th2 type mediators in the WT mice, while in the HDC KO mice the expression was polarized toward a Th1 response. According to our results we can suggest that the possible causes of the reduced asthma symptoms in the HDC KO mice may be the imperfect mast and eosinophil cell system and an altered immune response to OVA provocation and challenge.

[50] P

The Tuscan Centre for Pharmacovigilance: a regional centre for the diagnosis, management and prevention of Adverse Drug Reactions. The case of anti-histamines

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Adverse drug reactions (ADRs) are considered as one among the leading causes of morbidity and mortality and spontaneous reporting of ADRs has become an important component of monitoring and evaluation activities performed in health care systems. Anti-histamines are a category of drugs usually considered as safe. The most reported side effect is CNS sedation, an event that is known to vary between histamine H_1 receptor antagonists and between patients. New generation anti-histamines, such as desloratadine and levocetirizine have been reported to lack the CNS-depressant effects associated with first-generation antihistamines at routine clinical doses and to be associated with less side effects than older drugs of the same class.
Starting from January 2006, Tuscan County activated a Regional System of Pharmacovigilance to assist health care professionals in the diagnosis, management and prevention of ADRs. Aim of the present study was to evaluate the impact of the Tuscan Centre for Pharmacovigilance on ADR reporting, with particular reference to antihistamines. In the first year of activity of the Centre (January 1st - December 31th, 2006), the number of ADRs reported in Tuscany showed a 113% increase (vs a national variation of 14%). In particular, with regard to anti-histamines, the Italian Pharmacovigilance System recorded 28 ADRs during 2005 and 26 during 2006 (-7,2%), while in Tuscany the number of reports increased in the same period from 4 to 7 (+75,0%). No fatal case was observed; 67,9% of ADRs were reported as "mild" and 50% of ADRs reported as "severe" (among them, syncope and anaphylactic shock) were associated with clorfeniramine. Nevertheless, 75% of reported ADRs, although mainly of a mild severity (mainly nausea, dizziness, sedation and other CNS symptoms), were associated with new generation antihistamines, namely desloratadine and levocetirizine.
Data from Italian spontaneous signaling do not support the hypothesis that newer antihistamines are devoid of side effects typical of the "classical" drugs; nevertheless further studies conducted with an active pharmacovigilance design (such as prescription event monitoring) are needed to assess whether this phenomenon is clinically relevant or not.

[51] P

A new two-step procedure for the purification human peripheral blood basophils to near homogeneity

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Basophil research has long been hampered by the lack of an easy and effective purification protocol. Major progress was made in the past decade when immunomagnetic beads, which could be used for negative selection of basophils, became available. With this technology, it became possible for the first time to obtain homogeneous basophil preparations which could be used e.g. for signal transduction studies. Despite these advances, currently available protocols are dependent on specialised equipment such as elutriators, or are generally based on time-consuming, three-step purification protocols. Long purification times involving prolonged handling of basophils under non-physiological conditions, are more likely to result in potentially skewed gene expression and mediator release patterns, such as increased spontaneous histamine release.
We therefore devised a reproducible, rapid two-step method for the purification of basophils from small quantities of peripheral blood within 1.5 hours. Heparinized blood samples were first subjected to centrifugation in Hetasep® followed by negative selection using immunomagnetic beads. Basophil morphology and purity was assessed by May-Grünwald staining. IgE-mediated histamine releases were analysed spectrofluorometrically and cytokine production by RT-PCR. CD203c and CD63 expression were measured using flow cytometry. Basophils were enriched close to homogeneity in most cases with a mean purity of over 99% (range 97 - 100%, n=18) and a mean recovery of 75.6 (range 39 - 100%), with excellent viability. The purification procedure gave rise to basophils with normal functional responses to anti-IgE regarding histamine release and IL-4 mRNA expression. Moreover, constitutive cell-surface CD203c/CD63 expressions were not elevated comparing basophils in whole blood preparations with those that had been purified. The rapidity and reproducibility of this method will facilitate the employment of basophils in high-output ex-vivo studies.

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Ethical guidelines. Clinical studies must be performed in accordance with the "Declaration of Helsinki" and its amendment in "Tokyo and Venice". Experiments causing pain or discomfort to animals must be performed according to the guidelines of the International Association for the Study of Pain as published in Pain 1983;16:109-110. In accordance with these guidelines, authors performing such experiments must justify explicitly that the procedures used are scientifically necessary and that the minimum possible pain or stress has been imposed on the animals. Authors should also indicate whether the experimental work was reviewed by an ethical committee or its equivalent.

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Heaney LG, Cross LJM, Stanford CF, Ennis M. Substance P induces histamine release from human pulmonary mast cells. Clin Exp Allergy 1995;25:179-86.
Skidmore IF, Vardy CJ. The mediators of bronchial asthma and the mechanism of their release. In Saxena PR, Elliot GR, eds. Pathophysiology and Treatment of Asthma and Arthritis. Agents and Actions Suppl. vol. 14. Basel: Birkhäuser, 1984:33-48.

Siegel S, Nonparametric Statistics for the Behavioral Sciences. Tokyo: McGraw-Hill-Kogakusha, 1956:116-27.

For further examples see: N Engl J Med 1991;324:424-8.
Journal abbreviations should correspond to those in the World List of Scientific Periodical. Where possible, references should be easy to find.
A paper which has been accepted for publication but which has not yet appeared may be cited in the reference list with the words "in press" after the abbreviated name of the journal.

Unpublished results, papers in preparation and personal communications must be mentioned on the text ONLY; they are not to be included in the reference list. Personal communications may only be used when written authorisation from the communicator is submitted.

Illustrations and legends should, in all cases, be self-explanatory without reference to the text, numbers being given unobtrusively on the back of all submitted figures.

Tables should each be given on a separate sheet and prepared for use in a single column (8 cm wide) or for page width (16 cm). Each table must be numbered consecutively with Arabic numerals in italics and a brief descriptive caption should be given. The caption, headings and footnotes should be separated from each other and from the body of the table by horizontally ruled lines. Vertically rule lines must never be used. References to statistical significance may be made by using superscript asterisks. Otherwise, and where statistical significance is indicated together with footnotes, references should be made by using superscript lower case letters.

Figures should be suitable for reduction to fit a single column, 8 cm wide or at most a double column, maximally 16 cm wide. Original drawings in black ink or good photographic copies of original drawings should be submitted. Lettering should be not less than 4 mm high and lines not less than 0.4 mm thick on original drawings. Symbols should be not less than 2.5 mm in diameter, preferably chosen from: ● ┆ □○□

Photographs and micrographs should be printed on glossy paper. the size should be larger than, but not more than twice as large as the final size in the journal (i.e. 8 or 16 cm). Lettering should be added directly to the micrographs by the author.
Legends should be typed on a separate sheet(s) of paper paginated as part of the paper.

Abbreviations. The excessive use of abbreviations in the text is strongly discouraged. All abbreviations should be defined when first used by placing them in brackets after the full term. Symbols for physical units should adhere to the International System of Measurements and Units. Examples of these and of chemical and biochemical abbreviations and nomenclature may be found in Biochem J 1975;145:1-20 or in Br J Pharmacol 1984;81:3-10.

Submission of manuscripts
Please send the original and 1 paper copy of your manuscript together with a copy on disc. Texts on disc should be delivered in IBM PC format. They should be saved and delivered in two separate versions: with standard format as offered by your word processing program (give details of the program and version on the disc) and in Rich Text Format.
Hard copy originals of illustrations must be 200% of printed size.
Digital drawings, graphs etc should be submitted in JPG, GIF or TIF.

The International Anthem of the European Histamine Research Society

CHORUS: For it´s mine, for it´s mine,
Decarboxylated Histidine.
We´ve extracted you and weighed you.
By the living gut assayed you.
But we've yet to find our function - **Histamine!**

1. We talk of toxicosis / migraine, shock or halitosis
Singing Histaminosis all the day.
Trauma, burns and inflammation / headache, pain and constipation,
Singing Histaminosis all the day.

2. You give asthmatic wheezes / the allergic sneezes,
Singing Histaminosis all the day.
Though obscure as yet, the fact is / you´re involved in anaphylaxis,
Singing Histaminosis all the day.

3. Since the time of Dale and Barger / your files are longer, larger
Singing Histaminosis all the day.
The control of circulation / then gastric stimulation,
Singing Histaminosis all the day.

CHORUS

4. Mast cells by the dozen / and basophils, your cousin,
Singing Histaminosis all the day.
They come and they go / fluctuate to and fro,
Singing Histaminosis all the day.

5. We heard a lot of groaning / from the upstart, Serotonin,
Singing Histaminosis all the day.
Down with 5-hydroxytrypta / and up with good old hista,
Singing Histaminosis all the day.

6. Each year we meet in May / to concentrate and play,
Singing Histaminosis all the day.
What luck to have such friends / to cater for our trends,
Singing Histaminosis all the day.

CHORUS

7. In nineteen seventy two / to Paris we all flew,
Singing Histaminosis all the day.
Then Marburg upon Lahn / where Wilfried kept us calm,
Singing Histaminosis all the day.

8. Copenhagen as next year / the Mermaid to cheer,
Singing Histaminosis all the day.
In nineteen seventy five / Florence kept us alive,
Singing Histaminosis all the day.

9. To Paris for the next / to hear a new text,
Singing histaminosis all the day.
In nineteen seventy seven / London, it was Heaven,
Singing Histaminosis all the day.

CHORUS

10. Then Lodz with great care / we learned a lot there,
Singing Histaminosis all the day.
In nineteen seventy nine / to Stockholm this time
Singing Histaminosis all the day.

11. Then to Budapest we went / with Susan on the scent,
Singing histaminosis all the day.
West Germany again / for Hannover by name,
Singing Histaminosis all the day.

12. In nineteen eighty two / to Bled we all flew,
Singing Histaminosis all the day.
Then Brighton to the fore / with sea breezes by the shore,
Singing Histaminosis all the day.

CHORUS

13. And in nineteen eighty four / back in Florence like before,
Singing Histaminosis all the day.
Then in Aachen eighty five / Charlemagne became alive,
Singing Histaminosis all the day.

14. Then in Odense in Spring / in the Castle we did sing,
Singing Histaminosis all the day.
And then Czecho was the next / with our Rado at his best,
Singing Histaminosis all the day.

15. G.B. West was then cheered / for the ten years we´d been steered,
Singing Histaminosis all the day.
To Copenhagen again / we´re invited there by Svend
in the year eighty eight in lovely May.

CHORUS

16. And in nineteen eighty nine / and it was also fine,
we´re in Holland for the very first time.
To Kuopio in Finland / to the beautiful, but cold land,
we were watching the Finnish chopping wood.

17. Then to Marburg we returned / ninety one and also learned
that histamine in surgery´s not good.
Next year we met again / Manuel in sunny Spain,
Singing ai, ai and olé all the way.

18. Then with Eddy on the Rhine, we had more beer than wine,
Singing histaminosis all the day.
To Zsuzsanna ninety four / we went back to Danube shore,
Singing Histaminosis all the day.

CHORUS

19. Then with Igor ninety five / and the Volga was alive
And we entered the Russian Golden Ring.
In Antwerpen ninety six / Frans did show us a few tricks,
Singing Histaminosis all the day.

20. To Sevilla, once again / we all met in lovely Spain,
Singing Histaminosis all the day.
To Agnieszka ninety eight / back in Poland it was great,
Singing Histaminosis all the day.

21. Then to Lyon ninety nine / and Histamine´s still mine
Singing Histaminosis all the day.
New Millenium in Rome / Bruno made us all feel home
Singing Histaminosis all the day.

CHORUS

22. Pertti took us on a boat / we and Histamine could float
So to Turku we came two thousand one.
Andras called two thousand two / and to Eger did we go
A Hungarian meeting once again.

23. In the year two thousand three / we could lots of tulips see
Now Henk Timmerman was host in Amsterdam
Back to Germany next spring / and with Helmut did we sing
Singing Histaminosis all the day.

24. To lovely Bled we returned / and then once again we learned
That Histamine still lives two thousand five.
Then to Delphi we all came/and found Histamine the same
With Catherine in Greece two thousand six.

CHORUS

25. Returned to Florence the next year / For the third time we were here
And for us Emanuela made the day!
Let us sing this song together / Histamine will last forever
Singing Histaminosis all the day.

CHORUS

Index of authors