AVE Allergieverein in Europa e.V.
20. Jahrestagung

Hochschule Fulda
University of Applied Sciences

PROGRAMME AND ABSTRACTS

XXXVIIIth Meeting
European Histamine Research Society

Fulda, Germany **13-16 May 2009**

Herausgeber im Eigenverlag:

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CONTENTS

PREVIOUS EHRS ANNUAL MEETINGS

1972	Paris	1985	Aachen	1998	Lodz
1973	Marburg	1986	Odense	1999	Lyon
1974	Copenhagen	1987	Strbske Pleso	2000	Nemi (Rome)
1975	Florence	1988	Copenhagen	2001	Turku
1976	Paris	1989	Breda	2002	Eger
1977	London	1990	Kuopio	2003	Noordwijkerhout (Amsterdam)
1978	Lodz	1991	Marburg		
1979	Stockholm	1992	Malaga	2004	Bergisch-Gladbach
1980	Visegrád	1993	Cologne	2005	Bled
1981	Hannover	1994	Budapest	2006	Delphi
1982	Bled	1995	Moscow	2007	Florence
1983	Brighton	1996	Antwerp	2008	Stockholm
1984	Florence	1997	Seville		

WELCOME

Dear colleagues and friends,

We are happy to welcome you back to Germany –this time to the baroque City of Fulda– for the XXXVIIIth EHRS meeting 13th – 16th May 2009. The congress venue is in the centre of Fulda at the MARITIM Hotel.
This year the Allergie-Verein in Europa e.V. (AVE) will celebrate its 20th anniversary and is invited to organize a special joint session during the EHRS meeting.

Fulda is in the centre of Germany, about 100 km north east of Frankfurt am Main. It was founded by St Boniface 1250 years ago and in its old university students are still studying Catholic theology. With a population of about 100,000 Fulda is a friendly and quiet city in the middle of the countryside which believes in preserving traditions and caring about the surrounding natural landscape – the *Rhön*. The historical buildings include e.g. the Benedictine Monasteries and Catholic Convents, the roman *Michaels-Kirche* and the baroque Town Hall.
The congress venue is located in the typical baroque *Orangerie.* From here you are close to the *Cathedral* and also to the old town with many bars, restaurants, shopping centres and typical German *Kneipen.*
This year's meeting will be organized together with the *Allergie-Verein in Europa (AVE).* The *Fachbereich Oecotrophologie* looks forward to supporting the meeting not only by providing scientific issues but also organizing good music and great *Rhöner* cuisine.

Friedhelm Diel

CONGRESS VENUE
Maritim Hotel Fulda

HOST INSTITUTE
Dept Nutrition, Food and Consumer Sciences at the University of Applied Sciences HS Fulda

PRESIDENT EHRS
Ass. Prof. Dr. Anita Sydbom; Karolinska Institutet Stockholm, Sweden

PRESIDENT AVE e.V.
Dr. Eva Diel; Institut für Umwelt und Gesundheit IUG, Fulda

CHAIRMAN OF THE MEETING
Prof. Dr.-Ing. Friedhelm Diel; Dept Nutrition, University of Applied Sciences HS Fulda

ORGANISING COMMITTEE
Friedhelm Diel (Chairman), Eva Diel, Madeleine Ennis, Crispin Gigante Pérez,
Roman Khanferyan, Klaus-Dieter Koch, Cathleen Krieg, Frank Puin, Holger Stark,
Anita Sydbom (president EHRS), Heike Weisser

EXECUTIVE ASSISTANCE
Violetta Hames, Angelika Kejr, Jennifer Mages, Ursula Zimmermann

ABSTRACT EVALUATION AND BURSARY SELECTION COMMITTEE
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Patrizio Blandina
Paul Chazot
Friedhelm Diel
Bernie Gibbs
Gill Sturman
Ekaterini Tiligada

ARTHUR A HANCOCK YOUNG INVESTIGATOR AWARD JURY
Fred Pearce (Chairman); Marlon Cowart; Hubert Schwelberger

AVE YOUNG INVESTIGATOR AWARD JURY
Friedhelm Diel (Chairman); Roman Khanferyan; Per Stahl Skov; Hans Schubert

POSTER JURY
Walter Schunack (Chairman)
Hugo Boonen
Nicholas Carruthers
Agnieszka Fogel
Tatjana Irman-Florjanc
Jerzy Jochem
Emanuela Masini
Radomir Nosal

GENERAL INFORMATION

Registration Desk:
The registration desk will be open

Wednesday	15.00 – 22.00	Foyer Maritim Hotel
Thursday	08.00 – 18.00	"
Friday	08.00 – 13.00	"
Saturday	08.00 – 13.00	"

Congress website:
www.ehrs-2009.de
E-mail and internet services is provided at the Maritim Hotel

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 2 min, followed by a 3 min discussion. The posters will be on display throughout the meeting until Saturday morning. We encourage the presenters to stand by their posters during the interval and the poster session. The maximum poster size is 120 x 80 cm (h x w). 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. Prizes will be announced at the Farewell Dinner.

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. A PC equipped with MS PowerPoint for Data Projection will be available in the Conference Venue. The projector is only PC and NOT Mac compatible, except for Mac software after 2005. The presentation should be brought in a USB pen saver, saved in a file as: Name_of_presenter.ppt

Accommodation information

Contact for students
Tel: +49 661 9640 105
Fax: +49 661 9640 189
eMail: Maria Campuzano or visit http://www.hs-fulda.de/io

Contact Tourismus-Fulda
Frau Schrimpf und Frau Müller
Tel.: +49 661 102 181 2
Fax.: +49 661 102 281 1
Mail: tourismus@fulda.de
Website accomodation (english): http://www.tourismus-fulda.de/ehrs-en

Transportations
Every 2 hours leaves high velocity train (IC) FRA airport station to Fulda. After arrival at Fulda main station take a Taxi (5 min) to congress venue (Hotel Maritim).

Train: Deutsche Bahn

SOCIAL PROGRAM

Wednesday 09-05-13		
19.30-22.00	Get together party Drinks and tapas	Apollo Saal
Thursday 09-05-14		
10.30-16.30	Excursion for accompanying persons to Rasdorf "Point alpha", historical sight-seeing in the neighbourhood with lunch	
20.00-21.00	Concert Collegium musicum in the Marmorsaal	Baroque town hall
Friday 09-05-15		
10.30-12.30	Down town shopping and sight-seeing	Fulda
14.30-	Excursion starting at MARITIM Hotel incl. coffee and German cake	Fasanerie
	Dinner, music and dancing	Museums Keller
Saturday 09-05-16		
20.00	Farewell dinner (formal but not essential)	Maritim restaurant Honours - speeches - music

THE PROGRAM AT A GLANCE

Wednesday 13.05.09

15.00- Arrival – check in – registration
15.30- EHRS council meeting followed by COST meeting (extended steering committee)
19.30-22.00 Welcome party

Thursday 14.05.09

8.30-9.00 Opening ceremony
9.00-9.45 New Honorary Members – ceremony
9.45-10.30 Plenary lecture: **KH Friedrich - *Roles of transcription factor STAT3 in cancer and immune disorders***
10.30-11.00 Coffee break
11.00-12.00 Oral session I *Neurological and Histamine Responses*
12.00-13.00 Oral session II *Anti-histamines and Inflammatory Processes*
13.00-14.00 Buffet lunch
14.00-14.30 Nina Grosman lecture: **A Falus** – *Histamine and the "omics" era*
14.30-15.00 Plenary lecture: **M Schmelz - *Peripheral neuro-immune interactions modulate itch and pain***
15.00-16.00 Oral session III *Histamine (H3/H4)Receptors*
16.00-17.15 Poster 1 *Histamine Metabolism, Cellular Processes and Signal Transduction* Coffee
16.00-17.30 Poster 2 *Histamine Receptors, H4 receptor, Ligands*
17.30-18.45 Poster 3 *Allergotoxicology and Clinical Aspects, Immunology and Cancer*
17.15-18.45 Poster 4 *Neurological Effects, Histaminergic Responses, Analytical Approaches*
18.45-20.00 Poster Committee and free time for dinner (individual)
20.00-21.00 Greetings from the Mayor and Collegium musicum Stadtschloss-Marmorsaal

Friday 15.05.09

8.30-9.30 GB West lecture: **F Melchers - *Flexibilities within and around hematopoietic cell development***
9.30-10.30 Oral session IV *Gastrointestinal Tract and Lung Responses*
10.30-11.00 Coffee break
11.00-12.00 Oral session V *Miscellaneous*
12.00-13.30 Oral session VI *Histamine and Cell Responses*
Special lectures: **R Leurs, PS Skov, R Thurmond**
13.30-14.30 Buffet lunch (for those not taking part in AVE session)
14.30- Excursion start (for those not taking part in AVE session)
13.30- Lunch conference 20th AVE ceremonies – alternatively: excursion to the Rhön
14.00-14.30 AVE-Fest-Vortrag: **J Kleine-Tebbe - *Basophils – clinical aspects***
14.30-15.15 Round table: ***Histamine and Allergy***
15.15-16.00 General Assembly AVE
16.00- Excursion for AVE group and dinner at the old University of Fulda

Saturday 16.05.09

9.00-9.30 W Schmutzler lecture: **B Gibbs** - *Basophils – basic science aspects*
9.30-10.00 Plenary lecture: **E Pap** - *Histamine and pregnancy*
10.00-10.30 Coffee break and final poster viewing
10.30-12.00 Art A Hancock Young Investigator Award Symposium
12.00-13.00 Symp: ***MCs Status quo & quo vadis*** part I
U Blank, F Levi-Schaffer, M Maurer, G Nilsson
13.00-14.00 Buffet lunch
14.00-14.30 MC Symp part II
14.30-15.00 COST lecture **Katherine Tiligada**
15.00-15.30 Coffee break
15.30-16.00 Poster committee
16.00-18.00 General Assembly EHRS
20.00- Farewell dinner

Sunday 09-05-17

check out – departure

EHRS AVE 2009 **Scientific Program**

Wednesday
13.05.09

15.00-	Arrival - check in - registration
15.30-17.30	EHRS council meeting
17.30-19.30	Extended steering committee – COST-meeting
19.30-22.00	Reception and Welcome party

Thursday
14.05.09

8.30-9.00	**Opening ceremony** **Friedhelm Diel** (Chairman of the Meeting) **Roland Schopf** (President University of Applied Sciences HS Fulda 1998-2008) **Anita Sydbom** (President EHRS) Distribution of bursaries
9.00-9.45	**New Honorary Members - ceremony** **Madeleine Ennis (UK)** – introduced by **Fred Pearce (UK)** **Fred Pearce (UK)** – introduced by **Madeleine Ennis (UK)** **Bruno Mondovi (It)** – introduced by **Pierfrancesco Mannaioni (It)**
9.45-10.30 L1	**Plenary lecture (Sponsored by Merck Serono)** **Karlheinz Friedrich (Ger)** - ***Roles of transcription factor STAT3 in cancer and immune disorders*** introduced by **Friedhelm Diel (Ger)**
10.30-11.00	Coffee break and Poster viewing
11.00-12.00	**Oral session I: Neurological and Histaminergic Responses** chaired by **Helmut Haas (Ger)** and **Patrizio Blandina (It)**
11.00-11.15 O1	Helmut L. Haas, Regis Parmentier, Sergej Kolbaev, Boris Klyuch, David Vandael, Oliver Selbach, Jian-Sheng Lin, Olga A. Sergeeva (Ger) **Thyrotropin-releasing hormone (TRH) causes arousal through excitation of histaminergic neurons**
11.15-11.30 O2	Peter van Ruitenbeek, Anke Sambeth, Annemiek Vermeeren, Simon N. Young, Willem J. Riede (Ne) **Effects of L-histidine depletion and L-tyrosine/L-phenylalanine depletion on sensory and motor processes in healthy volunteers**
11.30-11.45 O3	Leonardo Munari, Maria Beatrice Passani, Fernando Benetti, Daniele Nosi, Timothy A. Esbenshade, Marlon D. Cowart, Jorge D. Brioni, Paul Chazot, Patrizio Blandina (It) **ABT-239, an H3 receptor Antagonist, reveals heterogenecity among histaminergic neurons**
11.45-12.00 O4	Kristoffer Sahlholm, Johanna Nilsson, Daniel Marcellino, Kjell Fuxe, Peter Århem (Swe) **Withdrawn**
12.00-13.00	**Oral session II: Anti-histamines and Inflammatory Processes** chaired by **Pierfrancesco Mannaioni (It)** and **Roman Khanferyan (Rus)**
12.00-12.15 O5	H. Boonen, BPM Martens, I Boersma, D. Kolbach, G. Krekels , E. Suys, A De Deene, Y. Doffiny, C. Leys, J. Hercegova, P. Arenberger, J. Beetens (Bel) **A one-week treatment with oral VAPITADINE, a new non-sedative anti-histamine, releaves itch in patients with chronic idiopatic urticaria**
12.15-12.30 O6	Roman Khanferyan, N. Milchenko (Rus) **H3/H4 receptor ligands modulate IgE activity induced by histamine**

12.30-12.45 O7
Jeffery M. Cowden, Mai Zhang, Paul J. Dunford, Robin L. Thurmond (USA)
The Histamine H4 receptor (H4R) mediates Inflammation and Pruritus in Th2-dependent Dermal Inflammation

12.45-13.00 O8
Zsuzsa Iffiú-Soltész, Estelle Wanecq, Danielle Prévot, Sandra Grès, Christian Carpéné (Fra)
Histamine oxidation is mainly controlled by AOC3 amine oxidase in mouse adipose tissue

13.00-14.00 Buffet Lunch

14.00.-14.30 L2
Nina Grosman Memorial Lecture
chaired by **Marija Čarman-Kržan (Slo)** and **Friedhelm Diel (Ger)**
Andras Falus (Hun)– ***Histamine and the "omics" era***

14.30-15.00 L3
Plenary lecture
Martin Schmelz (Ger) – ***Histamine-dependent and –independent itch and pain***

15.00-16.00
Oral session III: Histamine (H3/H4) Receptors
chaired by **Madeleine Ennis (UK)** and **Marlon Cowart (USA)**

15.00-15.15 O9
Natasha Lethbridge, Andrew Medhurst, Paul L. Chazot (UK)
Spinal Histamine H4 receptor expression in a rat chronic inflammatory pain model

15.15-15.30 O10
Kerstin Sander, Yvonne von Coburg, Tim Kottke, Xavier Ligneau, Holger Stark (Ger)
Histamine H3 receptor antagonists with antipsychotic components

15.30-15.45 O11
M.C. Vinci, C. Lanzi, R. Mastroianni, C. Uliva, L. Cinci, D. Bani, R.L Thurmond , and E. Masini (It)
Selective H4 R antagonist prevents antigen-induced airway inflammation in guinea pig: a pivotal role of annexin-A1

15.45-16.00 O12
Marlon Cowart, Gin Hsieh, Marina Strakhova, Timothy Esbenshade, Jorge D. Brioni (USA)
Histamine H4 receptor antagonists A-943931 and A-987306: in vitro profiles, and efficacy in pain, inflammation, and itch models

16.00-17.15
Poster session 1 and coffee
Histamine Metabolism, Cellular Processes and Signal Transduction
chaired by **Jerzy Jochem (Pol)** and **Nicholas Carruthers (USA)**

P1
Metoda Lipnik-Stangelj, Polonca Ferk, Branka Wraber (Slo)
Histamine stimulates IL-1beta mRNA expression and IL-1beta secretion from cultured astrocytes

P2
Michal Pyzlak, Grzegorz Szewczyk, Dariusz Szukiewicz, Anna Szczesniak (Pol)
Histamine influence on apoptosis in trophoblast cell cultures

P3
María S Sáez, Nora A Mohamad, Eduardo Valli, Elena S Rivera, Mariel A Núñez, Alicia S Gutiérrez, Graciela P Cricco, Gabriela A Martín (Arg)
Histamine and the Invasive Phenotype in Irradiated PANC-1 Cells

P4
Jean Sainte-Laudy, Catherine Martin (Fra)
Use of lipid rafting for the analysis of leukocyte activation by flow cytometry

P5
Ingrid Skard, Zsuzsa Darvas, László Kőhidai, András Falus, Sára Tóth (Hun)
Is Histamine Dependent Downregulation Of Fibulin Universal Or Cell Line Specific?

P6
Anayansi Molina, Ivan Velasco (Mex)
Histamine increases neuronal clonogenicity and induces expression of deep cortical layer markers after differentiation of neural stem cells

P7 Hubert G. Schwelberger (Au)
Structural Organization of Mammalian Copper-Containing Amine Oxidase Genes

P8 Jutta Haefner, Cathleen Krieg, Inna Michel, Heike Weisser, Friedhelm Diel (Ger)
IL-23 suppresses IL-17E (IL-25) in sensitized human lymphocytes *ex vivo*

P9 Hans J Schubert (Ger)
The lymphocyte transformation test (LTT) in type IV Allergy to chemical substances

P10 Dimitrios A Kyriakidis, Marina C Theodorou, Ekaterini Tiligada (Gre)
Histamine Affects AtoSC Two-Component System-Mediated Chemotaxis in *Escherichia coli*

P11 Melanie M. Hoefer, Harald Illges (USA, Ger)
Ectodomain Shedding and C-terminal Fragment (CTF) Formation of Complement Receptor 2/CD21 in Health and Disease

P12 Egle Passante, Neil Frankish (Fra)
Deficiencies in elements involved in TLR4-receptor signalling in RBL-2H3 cells

16.00-17.30 **Poster session 2** and coffee

Histamine Receptors, H4 Receptor, Ligands

chaired by **Tatjana Irman-Florjanc (Slo)** and **Hugo Boonen (Bel)**

P13 Tobias Birnkammer, Anja Kraus, Hendrik Preuss, Günther Bernhardt, Stefan Dove, Sigurd Elz; Roland Seifert, Armin Buschauer (Ger)
Toward bivalent Acylguanidine-type ligands: highly potent and selective histamine H2 receptor agonists

P14 Dariusz Szukiewicz, Grzegorz Szewczyk, Tarun K Mittal, Witold Rongies, Slawomir Maslinski (Pol)
Involvement of histamine and histamine H2 receptors in nicotinamide-induced differentiation of human amniotic epithelial cells (HAEC) into insulin producing cells

P15 Patrick Igel, David Schnell, Günther Bernhardt, Roland Seifert, Armin Buschauer (Ger)
Tritium-labeled N1-[3-(1H-Imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294), a high affinity histamine H3 and H4 receptor radioligand

P16 Timothy A. Esbenshade, Thomas R. Miller, Ivan Milicic, Joy Bauch, Jia Du, Bruce Surber, Kaitlin E. Browman, Marlon D. Cowart, Jorge D. Brioni (USA)
[^{3}H]A-349821 is a Useful H_3 Receptor Antagonist Radioligand in Revealing *In Vivo* Receptor Occupancy of Cognitive Enhancing H_3 Receptor Antagonists

P17 Daniela Erdmann, Johannes Mosandl, Günther Bernhardt, Roland Seifert, Otto S. Wolfbeis, Armin Buschauer (Ger)
Antagonistic activity and selectivity of fluorescent histamine H2 and H3 receptor ligands

P18 Kamil J Kuder, Tim Kottke, Holger Stark, Xavier Ligneau, Jean-Claude Camelin, Roland Seifert, Katarzyna Kieć-Kononowicz (Pol)
Search for Novel, Highly Affine Histamine H3 Receptor Ligands with Fluorescent Properties

P19 Patrick Igel, Roland Geyer, Erich Schneider, David Schnell, Roland Seifert, Armin Buschauer (Ger)
2-CYANO-1-[4-(1H-IMIDAZOL-4-YL)BUTYL]-3-[(2-PHENYLTHIO)ETHYL]-GUANIDINE (UR-PI376): a potent and selective histamine H4 receptor agonist

P20 Tim Kottke, Erich Schneider, Roland Seifert, Holger Stark (Ger)
Functional characterization of (1H-IMIDAZOL-4-YL)ALKYL derivatives at histamine H4 receptor

P21 Susanne Mommert, Gitta Köther, Thomas Werfel, Ralf Gutzmer (Ger)
Expression and function of histamine H4 receptor on human memory Th-17 cells.

P22 Tünde Simon, Ivett Jelinek, Valéria László, András Falus (Hun)
Detection and Function of H4R in Spleen- derived Dendritic Cells

P23 Tadeusz Karcz1, Jadwiga Handzlik, Dorota Łażewska, Tim Kottke, Erich Schneider, Roland Seifert, Holger Stark, Katarzyna Kieć-Kononowicz (Ger)
Search for Histamine H4 Receptor Ligands in the Group of 4-Methylpiperazine Derivatives

P24 Kristine Roßbach, Holger Stark, Kerstin Sander, Manfred Kietzmann, Wolfgang Bäumer (Ger)
Histamine H3 receptor antagonist-induced pruritus can be inhibited by blockade of histamine H1 and H4 receptors

P25 Evangelia Zampeli, Konstantinos Kyriakidis, Ekaterini Tiligada (Gre)
Differential effects of H_1 and H_4 Receptor Antagonists on the Cartilage Histamine Content in Rats with Adjuvant Arthritis

P26 José Alfón, Noelia Ardanaz, Beatriz Gil, Alberto Fernández, Dolors Balsa, Lluís Gómez, Manuel Merlos, Julio Cortijo, Esteban Morcillo, Xavier Bartrolí (Esp)
UR-60427 a Novel H4 Receptor Inverse Agonist that Shows Good Efficacy in Rodent Asthma Models

P27 Kathleen Isensee, Xavier Ligneau, Jean-Claude Camelin, Marc Capet, Jean-Charles Schwartz, Holger Stark (Ger)
Novel Fluorinated Non-Imidazole Histamine hH3 Receptor Antagonists of the Diamine Class

P28 Vanina A Medina, Maximo Croci, Graciela P Cricco, Ernesto JV Crescenti, Rosa M Bergoc, Elena S Rivera (Arg)
Histamine H4 Receptor Ligand JNJ7777120 Inhibits Lung Metastases in MDA-MB-231 Xenograft Tumor-bearing Mice

17.30-18.45 **Poster session 3** and coffee

Allergotoxicology and Clinical Aspects, Immunology and Cancer

chaired by **Emanuela Masini (It)** and **Fred Pearce (UK)**

P29 Andreas Steneberg, Jasmin Kalus (Ger)
Biogenic amines – Histamine intolerance (HIT) diet

P30 Noelia Massari, Vanina A Medina, Graciela P Cricco, Maximo Croci, Elena S Rivera (Arg)
Histamine-mediated Biological Responses in WM35 Human Melanoma Cells

P31 Konturek PC, T. Brzozowski, SJ Konturek, V. Kukharsky, S. Kwiecien, R. Pajdo, M. Raithel (Pol, Ger)
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P32 Raithel M, Nägel A, deRossi Th, Straube S, Stengel Ch, Ottmann B, Kressel J, Hahn EG, Konturek P (Ger)
Plasma histamine (H) levels during adjunctive H1-receptor antagonist treatment with loratadine in patients (pts) with active Inflammatory Bowel Disease (IBD)

P33 Bernhard F. Gibbs (UK)
Recruitment and Activation of Human Basophils in Bullous Pemphigoid: Enhancement of IgE-Mediated Histamine Secretion by Blister Fluid

P34 Raithel M, Nägel A, Straube S, Stengel Ch, Ottmann B, Kressel J, Hahn EG, Konturek P (Ger)
Prospective randomised, placebo-controlled trial of additional loratadine treatment in Inflammatory Bowel Disease (IBD)

P35 P. Rzodkiewicz, E.Wojtecka-Lukasik, D. Szukiewicz, W. Schunack, S. Maslinski (Pol)
Antihistaminic drugs modify casein-induced inflammation

P36 Silke Kimpel, Benita Giera, Jürgen Kressel, Fred Buchwald, Hans-Wolfgang Schultis, Eckhart G. Hahn, Martin Raithel (Ger)
The measurement of leukotrienes in urine - diagnostic option in systemic mastocytosis?

P37 Hubert G. Schwelberger (Au)
Histamine Intolerance - A Metabolic Disease?

P38 Angelika Kejr, Crispin Gigante Pérez, Violetta Hames, Cathleen Krieg, Jennifer Mages, Nadja König, Friedhelm Diel (Esp, Ger)
Receptive Music Therapy (rMT) and Saliva Histamine Secretion

P39 V. Hames, L. Berthe-Corti, M. Focken, M. Nachtkamp, E. Diel, C. Krieg, F. Diel (Ger)
***Ex vivo*-Responses of Mixtures of Bacterial Wax esters in Sensitized (S) and Non-sensitized (NS) Human Blood Cell Incubates**

P40 Cathleen Krieg, Violetta Hames, Katharina Schudmann, Friedhelm Diel, Klaus-Dieter Koch (Ger)
Saliva Histamine Secretion after *Cynara scolymus* L. (Artichokes) oral challenge

P41 E.Wojtecka-Lukasik, K.Ksiezopolska-Orlowska, E.Gaszewska,O. Krasowicz-Towalska, P. Rzodkiewicz, D.Maslinska, D. Szukiewicz, S.Maslinski (Pol)
Cryotherapy decreased histamine level in blood of patients with rheumatoid arthritis

P42 D. Maslinska, M. Laure-Kamionowska, P. Rzodkiewicz, E. Wojtecka-Lukasik, D. Szukiewicz, M. Karolczak, S. Maslinski (Pol)
Histamine in pericarditis of children with congenital heart malformations.

P43 Michael Mahler, Margrit Fooke (Ger)
Detection of allergen-specific IgE using the ALLERG-O-LIQ System based on the Reversed-enzyme-allergo-sorbent-test

17.15-18.45 **Poster session 4** and coffee

Neurological Effects, Histaminergic Responses and Analytical Approaches

chaired by **Walter Schunack (Ger)** and **Agnieszka Fogel (Pol)**

P44 Damijana M. Jurič, Marija Čarman-Kržan (Slo)
Regulatory role of histamine in astrocytic NT-3 synthesis

P45 Tatjana Irman-Florjanc, Jerzy Jochem (Slo, Pol)
Histamine-induced cardiovascular effects in rats after repeated treatment with amitriptyline

P46 Katja Perdan-Pirkmajer, Katarina Černe, Mojca Krzan (Slo)
Histamine uptake into cultured neonatal rat astrocytes is affected by histamine metabolism

P47 Miriam Walter, Kerstin Sander, Xavier Ligneau, Jean-Claude Camelin, Jean-Charles Schwartz, Holger Stark (Ger)
Click chemistry on histamine H_3 receptor ligands and first metal containing antagonists

P48 Gin C. Hsieh, Prisca Honore, Pai Madhavi, Erica J Wensink, Prasant Chandran, Anita K Salyers, Jill M Wetter, Chen Zhao, Michael W Decker, Timothy A Esbenshade, Marlon D Cowart, Jorge D Brioni (USA)
The histamine H3 receptor as a potential antinociceptive target: Effects of selective H3 antagonists in several preclinical pain models and the involvement of noradrenergic systems

P49 Tiina-Kaisa Kukko-Lukjanov, Minnamaija Lintunen, Niina Jalava, Francisco R. Lopez-Picon, Hanna Lauren, Kimmo A. Michelsen, Pertti Panula, Irma E. Holopainen (Fin)
Severity of seizures and neuronal damage are enhanced in the 9-day-old histamine 1 receptor knockout mice

P50 Jun ZHANG, Jing-Ning ZHU, Lei YU, Hong-Zhao LI, Jian-Jun Wang (China)
Histamine selectively excites projection neurons in the cerebellar nuclei

P51 Beatrice YC Wan, Samuel Mann, El-Sayed K Assem, Charles M Marson (UK)
Effect of Syntheetic Antioxidants on H2O2 Guinea-pig Colon Smooth Muscle Contraction (GPCC)

P52 V.Markosov, N.Ageeva, F.Diel, R.Khanferyan (Rus)
Histidine and Histamine Concentrations in Wines

P53 Regina Modi (Ger)
Feed Microscopy as a Possible Instrument in Allergy Prevention

P54 Andreas Steneberg, Michaela Mayer, Ursula Zimmermann
Histamine restricted winemaking – from vintage to fining

P55 Sigurd Elz, Patrick Igel, Roland Geyer, Anja Kraus, Marc Kunze, Tobias Birnkammer, Armin Buschauer (Ger)
CIMETIDINE: A veteran H2-reseptor antagonist for the characterisation of novel potent ACYLGUANIDINE-type H2-receptor agonists

P56 Andrea Straßer, Hans-Joachim Wittmann, Karl-F. Deml, Sigurd Elz, Tobias Birnkammer, Roland Seifert (Ger)
Dual histamine H1- and H4-receptor ligands

P57 Anna Stasiak, Miroslaw Mussur, Mercedes Unzeta and Wieslawa Agnieszka Fogel (Pol)
CNS histamine in rat model of vascular dementia

P58 Edward Oganesyan, Vladimir Agadjanyan, Svetlana Mutzueva, Roman Khanferyan (Rus)
The development of aniallergic and antihistaminic agents among polyphenols with high antiradical activity

P59 Ivan Kodonidi, Denis Zolotych, Edward Oganesyan (Rus)
Antiallergic and immunomodulating activity of N -[heteryl-substituted] of 4-[oxopyrimidine]

P60 Rogier Smits, Iwan de Esch, Cindy van Dam, Obbe Zuiderveld, Gabriella Coruzzi, Maristella Adami, Gunnar Flik, Thomas Creemers, Rob Leurs (Ne)
A novel series of potent histamineH_4 receptor antagonists: Orally bioavailable QUINAZOLINE SULFONAMIDES with anti-inflammatory activity *in vivo*

P61	Almudena Pino-Ángeles, Aurelio A. Moya-García, Francisca Sánchez-Jiménez (Esp) **Computational Approach to Structure, Function and Specific Intervention on Mammalian Histidine Decarboxylase**
18.45	Poster committee chaired by **Walter Schunack (Ger)**
18.45-20.00	Free time for dinner (individual)
20.00-21.00	Greetings from the Mayor and Collegium musicum

Friday
15.05.09

8.30-9.30 L4	**GB West Lecture** **Fritz Melchers (Ger)- *Flexibilities within and around hematopoietic cell development*** introduced by **Walter Schunack (Ger)**
9.30-10.30	**Oral session IV: Gastrointestinal Tract and Lung Responses** chaired by **Hubert Schwelberger (At)** and **Ralf Gutzmer (Ger)**
9.30-9.45 O13	P.C. Konturek, SJ Konturek, M. Raithel, S. Kwiecien, G. Burnat, T. Brzozowski (Pol) **Histamine treated rats with occlusion of gastric artery and pylorus ligation develop gastric lesions progressing into chronic ulcerations. Gastroprotective effect of prostaglandins**
9.45-10.00 O14	John Ionescu (Ger) **Gut mediated pseudoallergic reactions in atopic and environmental patients**
10.00-10.15 O15	Bettina M. Jensen, Pernille M. Frandsen, Ellen Margrethe Raaby, Peter Oluf Schiøtz, Per S. Skov and Lars K. Poulsen (Dan). **Differences in activation patterns between peripheral blood and cord blood derived human mast cells**
10.15-10.30 O16	Suzanne Havard, Linda J Kay, Susan S Ishmael, Donald W MacGlashan, Peter T Peachell (UK) **Human lung mast cell 'releasability': role of syk**
10.30-11.00	Coffee break
11.00-12.00	**Oral session V: Miscellaneous** chaired by **Andras Falus (Hun)** and **Bernie Gibbs (UK)**
11.00-11.15 O17	Vanina A Medina, Juan P Prestifilippo, Maximo Croci, Rosa M Bergoc, Juan C Elverdin, Elena S Rivera (Arg) **Histamine Prevents Functional and Histological Alterations of Salivary Glands Exerted by Ionizing Radiation**
11.15-11.30 O18	El-Sayed K Assem, Samuel Mann, Beatrice YC Wan, Charles M Marson (UK) **Effect of some lipoic acid derivatives and other anti-oxidants on the histamine-induced contraction of guinea-pig tracheal smooth muscle**
11.30-11.45 O19	Jean Sainte-Laudy, François Machavoine, Michel Dy (Fra) **Inhibition of histamine release and intracellular protein synthesis by histamine high dilutions**
11.45-12.00 O20	Michael Mahler, Ralf Lucassen, Margrit Fooke (Ger) **Reliable rapid detection of allergen-specific and total IgE in the diagnosis of type I hypersensitivity using a novel allergy lateral flow assay (ALFA)**
12.00-13.30	**Oral session VI: Histamine and Cell Responses** chaired by **Per S Skov (Dan)** and **Holger Stark (Ger)**
12.00-12.15 O21	Susanne Diel, Robert Teachenor, Cornelis Murre (USA) **The Function of Transcription Factor E2A in CD8 Cell Regulation**

12.15-12.30 O22	Dariusz Szukiewicz, Michal Pyzlak, Aleksandra Stangret, Witold Rongies, Danuta Maslinska (Pol) **Decrease in expression of histamine H2 receptors by human amniotic epithelial cells (HAEC) during differentiation into pancreatic beta-like cells (PBLC)**
12.30-12.50 O23	**Per Stahl Skov (Dan)**– ***The clinical usefulness of basophil histamine release***
12.50-13.30 O24 O25	**R Leurs (Ne)**– ***New molecular insights in the H4R-receptor field*** **R Thurmond (USA)** – ***H4R-ligands – clinical aspects***
13.30-14.30	Buffet Lunch
14.30-	Excursion and dinner at the old University of Fulda
13.45-15.15	**Lunch conference 20th AVE ceremonies** chaired by **Eva Diel** (President AVE) Welcome – *Begrüßungen und Ehrungen*
13.45-14.00	HMWK and **Karim Khakzar** (President Hochschule Fulda) AVE Young investigator award
14.00-14.30 L5	AVE-*Festvortrag*: **Jörg Kleine-Tebbe (Ger)** – ***Basophils – clinical aspects*** introduced by **Norbert Bethge**
14.30-15.15	**Round table** moderated by **Madeleine Ennis (UK)** – ***Histamine and Allergy*** Hugo Boonen (Bel), John Ionescu (Ger), Roman Khanferyan (Rus), Jörg Kleine-Tebbe (Ger), Jean Sainte-Laudy (Fra), Hans Schubert (Ger), Per S Skov (Dan)
15.15-16.00	General Assembly AVE e.V. chaired by **Eva Diel**
16.00-	Excursion and dinner at the old University of Fulda (AVE-group)

Saturday
16.05.09

9.00-9.30 L6	**Plenary lectures** chaired by **Marija Carman Krzan (Slo)** and **Friedhelm Diel (Ger)** **Wolfgang Schmutzler Memorial Lecture** **Bernie Gibbs (UK)** – ***Basophils – basic science aspects***
9.30-10.00 L7	**Plenary lecture** **Erna Pap (Hun)** – ***Histamine and pregnancy***
10.00-10.30	Coffee break and final poster viewing – Poster scoring chaired by **Walter Schunack (Ger)**
10.30-12.00	**Art A Hancock Young Investigator Award Symposium** chaired by **Marlon Cowart (USA)** and **Fred Pearce (UK)**
O26	Kerstin Sander, Tim Kottke, Ewgenij Proschak, Yusuf Tanrikulu, Erich Schneider, Roland Seifert, Gisbert Schneider, Holger Stark (Ger) **Development of diaminopyrimidines as histamine H_4 receptor ligands – virtual screening and Scaffold optimisation**

O27	Tiina-Kaisa Kukko-Lukjanov, Minnamaija Lintunen, Niina Jalava, Francisco R. Lopez-Picon, Hanna Lauren, Kimmo A. Michelsen, Pertti Panula, Irma E. Holopainen (Fin) **Severity of seizures and neuronal damage are enhanced in the 9-day-old histamine 1 receptor knockout mice**
O28	Konstantinos Kyriakidis, Evangelia Zampeli, Ekaterini Tiligada (Gre) **Effect of the H_4 Receptor Antagonist JNJ7777120 on Histamine Levels of Peripheral Blood Vessels in Rats with Adjuvant Arthritis**
O29	Maria Gschwandtner, Kristine Rossbach, Wolfgang Bäumer, Manfred Kietzmann, Dorothea Dijkstra, Holger Stark, Thomas Werfel, Ralf Gutzmer (Ger) **Murine and Human Langerhans Cells Express a Functional Histamine H4 Receptor: Modulation of Cell Migration and Function**
O30	Natasha Lethbridge, Margaret Piggott, Andrew D Medhurst, Paul L. Chazot (UK) **Histamine H3 receptors in Lewy Body dementias: an autoradiographical study with [3H] GSK189254**
O31	Hilary S Whitworth, Xiaoying Zhou, Kathy J Bodey, Jane S Lucas, Andrew F Walls (UK) **Mast Cell and Basophil Markers for Predicting Severity of Allergic Reactions to Food**

12.00-13.00	Symp. ***MCs Status quo & quo vadis*** part I chaired by **Wilfried Lorenz (Ger)** and **Marcus Maurer (Ger)**
O32	**Stephan Bischoff (Ger) - *Withdrawn***
O33	**Uli Blank (Fra) - *Positive and negative role of mast cells in an experimental model of autoimmune glomerulobnephritis***
O34	**Francesca Levi-Schaffer (Isr) - *Mast cell interactions with their microenvironment in allergic inflammation***
13.00-14.00	Buffet Lunch
14.00-15.00	***MCs Status quo & quo vadis* part II** **Marcus Maurer (Ger) - *Mast cells and the skin***
O35	**Gunnar Nilsson (Swe) - *Regulation of mast cell survival in health and disease***
O36	**R.Nosál**, K.Drábiková, V.Jančinová, J.Králová, A Lojek, M.Číž (Slk)
O37	**On the interaction of H1-antihistamines with blood platelets and neutrophil leukocytes**
15.00-15.30 O38	**COST lecture** Ekaterini Tiligada (Gre) **Aims and Perspectives of the COST Action BM0806: Recent advances in histamine receptor H_4R research**
15.30-16.00	Coffee break
16.00-18.00	General Assembly EHRS chaired by **Anita Sydbom (Swe)**
20.00-	Farewell dinner
Sunday 17.05.09	Check out

L1

Roles of transcription factor STAT3 in cancer and immune disorders

Karlheinz Friedrich
Institute of Biochemistry, University of Jena Medical School, 07743 Jena, Germany

(STAT3) Signal Transducer and Activator of Transcription 3 is a regulator of fundamental cellular processes such as proliferation, differentiation and apoptosis.
It becomes activated through tyrosine phosphorylation by various cytokine and growth factor receptors and, upon dimerization and translocation to the nucleus, interacts with cognate binding sites in the DNA. In concert with other transcription factors and, dependent on the tissue context, it controls the expression of specific target genes.
In line with its essential activities, STAT3 malfunction has been associated with a multitude of diseases. Aberrant activation of STAT3 is observed in many hematopoietic cancers as well as in solid tumors. In fact, STAT3 can induce malignant cell transformation and act as a *bona fide* oncoprotein. Since it also appears to be involved in metastasis by driving the expression of invasiveness-related proteins such as matrix metalloproteinases, STAT3 is increasingly recognized as a potential prognostic marker and target for future cancer therapies.
The characterization of established and novel T cell subsets in recent years revealed that STAT3 is absolutely required for the generation of TH17 immune responses and, thus, is most probably a key player in the development of autoimmune disease. Consistent with this interpretation, a deficiency of TH17 cells and hyper IgE syndrome coincides with mutations in STAT3. STAT3 is probably also involved in the allergy-associated, mediated shift of cytokine production towards the TH2 pattern, since blocking the STAT signaling pathway prevents histamine-induced secretion of IL-13 by TH2 cells.

L2

Histamine research and the ´omics` era - Nina Grossmann Memorial lecture

András Falus
Dept. of Genetics, Cell/ and Immunobiology, Semmelweis University, Budapest 1089, Hungary

Systems biology represents an entirely new holistic approach in experimental biology. Due to availability of molecular databases, rapid development of high-throughput molecular technologies and the robust bioinformatic/bibliomic and pathway networking methodologies the contemporary biology got a revolutionary input. Obviously, genetic/genomic data at DNA level, mRNA and microRNA as well as protein expression combining with metabolomics influenced histaminology, as well. The lecture attempts to summarize genomic, transcriptomics, proteomics and metabolomics of enzymes involved in histamine metabolism, histidine decarboxylase, catabolizing enzymes, histamine receptors.

L3

Histamine-dependent and independent itch and pain

Martin Schmelz
Karl-Feuerstein Professorship, Dept. Anesthesiology Mannheim, University Heidelberg, 68167 Mannheim

Itch and pain are distinct sensations provoking opposing reflex patters, but there are complex interactions between them. The idea of a transition between itch evoking weak stimuli turning into pain upon increased stimulation intensity has been abandoned. Instead, the discovery of itch selective neurons in the periphery and in the spinal cord has clearly emphasized the specificity-theory of itch. The idea of two separate neuronal systems is in accordance with the antagonistic interactions between itch and pain: scratch-induced pain can abolish itch and analgesic opioids can generate itch.
Recent data show astonishingly similar mediators and mechanisms of neuronal sensitization in the periphery and the central nervous system in itch and pain: Proteinase-activated receptors (PAR-2) have been linked both to pain and itch. More recently, the important role of nerve growth factor (NGF) has emerged both in painful and pruritic diseases: The expression of nerve growth factor (NGF) is high in injured and inflamed tissues and activation of the NGF receptor, tyrosine kinase trkA, on nociceptive neurons triggers and potentiates pain-signalling by multiple mechanisms. Histamine-dependent itch has been the main experimental approach to induce pruritus in humans, but clinically histamine-independent types are more important. Thus, currently itch research is focussing on distinctions between histamine-dependent and histamine-independent nerve fibers, which could explain itch without accompanying axon reflex erythma. It can therefore be expected that also pruriceptive nerve fibers have different classes, as do the C nociceptors. Different classes of pruriceptors could also account for the various submodalities of pruritus reported by patients.
In summary, itch research has moved forward dramatically by identifying itch-selective neuronal pathways and mechanisms of sensitization in the periphery and the central nervous system. Based on the striking similarities of sensitization processes in chronic pain and itch analgesic therapies have already been successfully used in treating chronic itch. Future research can now be focussed on molecular targets to provide the basis for future antipruritic therapy.

L4

Flexibilities within and around hematopoietic cell development

Fritz Melchers
Biozentrum der Universität Basel, Basel, Max Planck Fellow, Lymphocyte Development Group, Max Planck Institute for Infection Biology, Berlin

All hematopoietic cell lineages can be generated from a single pluripotent hematopoietic stem cell (pHSC). Such a stem cell has five important properties: It can renew itself by symmetric or asymmetric division. It is pluripotent, hence can give rise to erythrocytes, megakaryocytes and platelets, to myeloid cells such as monocytes, macrophages, dendritic cells, osteoclasts, eosinophils, basophils, neutrophils and mast cells, and to lymphoid cells, such as natural killer cells and the various functional subpopulations of T and B lymphocytes. Upon transplantation a stem cell is capable of homing back to the bone marrow, its original site in the body. It can be reisolated from this bone marrow of the recipient mouse with the same self-renewing, pluripotent and bone marrow-homing properties, hence can populate and repopulate the host in a long-term reconstituting way. Finally its transplantation can protect a lethally irradiated host from death. These stem cells allow continuous production of all hematopoietic cell lineages most of which turn over with half-lives of a few days. During hematopoietic development and differentiation such so-called long-term pHSC's develop first into short-term pHSC that have lost the capacity to remain in the bone marrow for periods longer than 4 weeks, therefore give rise to one wave of pluri-lineage development after transplantation. From these cells myeloid/lymphoid progenitors, then myeloid and lymphoid progenitors, and thereafter progenitors of more specialized sublineages of myeloid and lymphoid progenitors and precursors develop. In the case of lymphoid development decisions to become either a natural killer cell, a T lymphocyte or a B lymphocyte is made. These developmental decisions are controlled by cell-autonomous signal transduction and gene expression programs, which, in turn, are influenced by cell-cell contact, and by cytokines and chemokines acting with receptors for such ligands which are provided by (mostly mesenchymally derived) stromal cells that form "niches" in which these developments take place. Changes in genes and molecules either of the hematopoietic or the environmental stromal cells can change the capacities and potencies of these cells.Pluripotent HSC's and many of the progenitors and precursors can now be maintained and grown in tissue culture, can be genetically modified and can be transplanted to test their hematopoietic potentials, allowing a more systematic analysis of the molecular programs and the cellular stages in hematopoietic development. Examples of such changes will be described. The presentation will also attempt a simplified description of the developmental pathways which generate cells involved in allergy.

L5

Basophils - Clinical Aspects

Jörg Kleine-Tebbe
Allergy & Asthma Center Westend; Private Practice Hanf, Herold & Kleine-Tebbe, Berlin, Germany

Despite a lack in understanding their physiological functions human basophils are actively involved in various immune responses including allergic inflammation awaiting further clinical definition of their role.
Basophil functions in human health and disease have been proposed after morphological and functional ex vivo studies, but also extrapolated from recent mouse studies. Fully equipped as a potent effector cell, basophils are involved in early as well as in late allergic responses, might drive inflammation during parasitic and viral infections, and could potentially serve as regulatory cells modifying innate and T helper type 2 driven immunity. Basophils appear in human skin, bronchial biopsies, nasal lavages under various conditions like atopic/contact dermatitis, asthma including fatalities and after allergen challenge procedures.
Even before the discovery of IgE human basophils served as preferred model of allergic immediate type hypersensitivity reactions and have been extensively studied in terms of immunological and non-immunological mediator and cytokine release representing a unique model for signal-secretion coupling. Subsequently, particular signaling pathways have been identified and IgE receptor regulation was demonstrated utilizing human basophils.
Due to their amplifying apparatus and high analytical sensitivity basophil histamine release, leukotriene production and surface marker expression (i.e. CD63, CD203c) have been used for diagnostic purposes demonstrating indirectly IgE-mediated sensitizations in allergic individuals as well as a read out for monitoring allergen specific immunotherapy and Anti-IgE injections in allergic asthma. However, variables indirectly effecting basophil functions like relationship between free IgE levels and receptor expression, the ratio of specific IgE level to total IgE level, and intrinsic cellular sensitivity have complicated simple conclusions from diagnostic or monitoring results based on human basophils.

L6

Basophils: basic science aspects - W Schmutzler lecture

Bernhard F Gibbs
Medway School of Pharmacy, University of Kent at Medway, Chatham Maritime, ME4 4TB, United Kingdom

Although basophils comprise of only a small fraction of circulating leukocytes there is increasing evidence to suggest a critical role for these cells as both effector cells and immunomodulators of allergic responses. Compared to their mast cell counterparts, basophils are generally 1-2 orders of magnitude more sensitive to IgE-mediated stimulation (e.g. allergens) and can release substantial quantities of histamine and LTC-4, as well as the Th2-type cytokines, IL-4 and IL-13. Recent evidence has now confirmed that these basophil-derived cytokines, in particular, support IgE synthesis and underlying atopy. Importantly, these mediators are also secreted following primary exposure to certain parasites (e.g. Schistosoma mansoni) and B cell superantigens, suggesting a role for basophils in innate immunity and in assisting developing Th2-type adaptive immune responses. In addition to these actions, IgE-dependent activation of basophils results in HIF-1alpha accumulation and VEGF synthesis, suggesting both a role for these cells in angiogenesis and adaptation to cellular stress. While we are only beginning to understand the pathophysiological functions of these cells, elucidation of key Fc<epsilon>RI-mediated signal transduction processes has led to novel therapeutic strategies for inhibiting mediator secretions. A potentially important discovery in this regard is the phosphatase SHIP, which downregulates PI 3-kinase signalling in both basophils and mast cells. Recent data shows that SHIP expressions may be increased, and the activity of basophils subsequently inhibited, by targeting receptors associated with SHIP recruitment (e.g. CD200R). Identifying the natural ligands for these inhibitory receptors and unravelling further inhibitory enzymes involved in basophil signal transduction may pave the way for new therapies for the treatment of allergic inflammation.

L7

Histamine and Pregnancy

Erna Pap*, *Éva Pállinger, András Falus
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Histamine has multiple functions in the process of pregnancy due to its vasoactive, differentiating and growth-promoting characteristics. Pre – and postimplantation events are accompanied by high histidine decarboxylase (HDC) enzyme activity. H1, H2 receptors and diamine-oxidase are co-expressed in both decidual and placental cells in humans. Furthermore, the expression of HDC is much higher in the placenta than in any other organ.
Asthma is the most common, potentially serious medical problem during pregnancy. Its prevalence is increasing, today it is between 4-7% worldvilde among pregnant women. The increased risk of complications can lead to preeclampsia, preterm delivery and low birth weight. The severity of asthma is changing during gestation, often in an unpredictable way.
It is a relatively recent finding that microvesicles (MV) represent a pivotal role in information transfer between cells. MVs are membrane covered small particles, derived from various cell types. Their possible diverse origine includes, among others, blood cells and even trophoblast cells, so MVs 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. This present overview aims to highlight some aspects of the relations between histamine and healthy and asthmatic pregnancy, underlining our new findings in respect to the microvesicular “regulation”.

Pap E, Falus A,. Mihályi D, Borck H, Diel F, Pállinger É. Histamine regulates placental cytokine expression – in vivo study on HDC knockout mice. *Placenta 2007,28: 239-244.*
Pap E, Pállinger E, Falus A, Kiss AA, Kittel A, Kovács P, Buzás EI. T lymphocytes are targets for platelet- and trophoblast-derived microvesicles during pregnancy. *Placenta 2008: 826-83.*
E. Pap, É. Pállinger, M. Pásztói, A. Falus. Highlights of a new type of intercellular communication: microvesicle-based information transfer . *Inflammation Research 2008: 1-8.*

O1

Thyrotropin-releasing hormone (TRH) causes arousal through excitation of histaminergic neurons

***Helmut L Haas*[1], *Regis Parmentier*[2], *Sergej Kolbaev*[1], *Boris Klyuch*[1], *David Vandael*[1], *Oliver Selbach*[1], *Jian-Sheng Lin*[2], *Olga A Sergeeva*[1]**
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The firing of histaminergic tuberomamillary nucleus (TMN) neurons controls arousal and attention, being active during waking, silent during sleep. Thyrotropin-releasing hormone (TRH) promotes arousal and combats sleepiness associated with narcolepsy. Single-cell RT-PCR (scRT-PCR) demonstrated variable expression of the two known TRH receptors in the majority of TMN neurons. TRH increased the firing rate of most TMN neurons. This excitation was abolished by the TRH receptor antagonist chlordiazepoxide. TRH depolarized TMN neurons directly without changing their input resistance. This effect reversed at the potential typical for nonselective cation channels. The potassium channel blockers barium and cesium did not influence the TRH-induced depolarization but TRH effects were antagonized by inhibitors of the Na+/Ca2+ exchanger, KB-R7943 and benzamil. The frequency of GABAergic spontaneous inhibitory postsynaptic currents was either increased (TTX-insensitive) or decreased (TTX-sensitive sIPSCs) by TRH, indicating a heterogeneous modulation of GABAergic inputs by TRH. Facilitation but not depression of sIPSC frequency by TRH was missing in the presence of the kappa-opioid receptor antagonist nor-binaltorphimine. Montirelin, a TRH analogue, induced waking in wild type mice but not in histidine decarboxylase knockout mice lacking histamine. Inhibition of histamine synthesis by (S)-alpha-fluoromethylhistidine also blocked the arousal effect of montirelin in WT mice. Thus direct receptor-mediated excitation of rodent TMN neurons by TRH demands activation of nonselective cation channels as well as electrogenic Na+/Ca2+ exchange. Our findings indicate a key role of the brain histamine system in TRH-induced arousal.

O2

Effects of L-histidine depletion and L-tyrosine/L-phenylalanine depletion on sensory and motor processes in healthy volunteers

***Peter van Ruitenbeek*, Anke Sambeth, Annemiek Vermeeren, Simon N Young, Willem J Riede**
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Animal studies have shown that histamine plays a role in cognitive functioning and that H_3-receptor antagonists, which increase histamine function through presynaptic receptors, improve cognitive performance in models for cognitive deficits seen in clinical disorders. In order to test such new drugs in humans, a model for cognitive impairments following low histamine functioning may be useful. Previous studies have shown that H_1-antagonsists may be limited as a model. This present study is the first to evaluate whether the depletion histamine's precursor L-histidine is effective in altering measures associated with histamine in humans and the behavioural and electrophysiological (event-related-potentials) effects.
Seventeen healthy volunteers completed a 3-way, double blind, cross-over study with L-histidine depletion, L-tyrosine/L-phenylalanine depletion (active control) and placebo as treatments. The interactions with task manipulations in a choice reaction time task were studied. Task demands were increased using visual stimulus degradation and increased response complexity. In addition, subjective and objective measures of sedation and critical tracking task performance were assessed.
Measures of sedation and critical tracking task performance were not affected by treatment. L-histidine depletion was effective and enlarged the effect of Response Complexity as measured with the response locked Lateralized Readiness Potential onset latency.
L-histidine depletion affected response- but not stimulus related processes. This is in contrast to the effects of H_1-antagonists which were previously found to affect primarily stimulus related processes. L-histidine depletion is promising as a model for histamine based impairments. However, future studies need to confirm these effects.

O3

ABT-239, an H3 receptor Antagonist, reveals heterogenecity among histaminergic neurons

Leonardo Munari*[1], *Maria Beatrice Passani*[1], *Fernando Benetti*[1], *Daniele Nosi*[2], *Timothy A Esbenshade*[3], *Marlon D Cowart*[3], *Jorge D Brioni*[3], *Paul L Chazot*[4], ***Patrizio Blandina[1]**

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Neuronal histamine (HA) is released from axon varicosities innervating the entire brain, and originating within a single source, the tuberomammillary nucleus (TMN). HA neurons react selectively to pharmacological stimuli, and form functional groups according to their regional projections [1,2]. This implies independent functions for subsets of histaminergic neurons. In the present study we used microdialysis in freely moving rats to investigate whether ABT-239 discriminates groups of HA neurons impinging on different brain regions. SD rats were implanted with one probe in the TMN, and another 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. Significant effects were determined by ANOVA/Bonferroni/Dunn test. Basal HA release from all regions was stable, ranging 0.04-0.08 pmol/15min (N=28). I.p. administration of ABT-239 (1 mg/kg) increased HA release from TMN up to about 80%. ABT-239 (10µM), infused for 60 min into the TMN, increased HA release from the TMN, NBM and PFC, but not from the DS nor NAc. These results suggest that H_3R blockade does not activate all HA neurons. To add strength to this observation, we examined H_3R distribution on HA neurons. Hypothalamic slices were double-labelled with antibodies against H_3Rs and histidine decarboxylase (HDC). Confocal analysis showed that HDC-positive neurons form two populations, strongly or weakly immunopositive for H_3R antibodies, respectively, thus suggesting that not all HA neurons carry H_3Rs. 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] Giannoni P *et al,* 36th EHRS Meeting, 9-12/5/2007, Firenze, I.

O4

Voltage-insensitivity of proxyfan activating GIRK channels via the histamine H_3 receptor

Kristoffer Sahlholm*, *Johanna Nilsson, Daniel Marcellino, Kjell Fuxe, Peter Århem
Karolinska Institutet, Dept. of Neuroscience, Stockholm, 171 77, Sweden

Withdrawn

O5

A one-week treatment with oral VAPITADINE, a new non-sedative anti-histamine, releaves itch in patients with chronic idiopatic urticaria

***H Boonen*[1], *BPM Martens*[2], *I Boersma*[3], *D Kolbach*[4], *G Krekels*[5] , *E Suys*[6], *A De Deene*[7], *Y Doffiny*[8], *C Leys*[9], *J Hercegova*[10], *P Arenberger*[11], *J Beetens*[12]**

[1]Private Practice Dermatology, Geel, Belgium,
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[5]Catherina Ziekenhuis, Dept of Dermatology, Eindhoven, The Netherlands,
[6]Private Practice Dermatology, Kortrijk, Belgium,
[7]Private Practice Dermatology, Lier , Belgium,
[8]Private Practice Dermatology, Marchienne-au-Pont, Belgium,
[9]Private Practice Dermatology, Sint Truiden, Belgium,
[10]Private Practice Dermatology, Praha, Czech Republic,
[11]Dermatovenerology Out-Patient Clinics, Praha, Czech Republic,
[12]Barrier Therapeutics NV, Geel, Belgium

Objective: To evaluate the efficacy of oral vapitadine 60 mg od for 1 week on the alleviation of itch symptoms in subjects with chronic idiopatic urticaria (CIU)
Methods: This randomized, multicenter, double-blind, placebo-controlled, exploratory trial included 44 subjects with CIU. After a run-in period of 3 to 7 days, subjects with confirmed diagnosis of CIU were randomly assigned to vapitadine oral solution or placebo *od* for 6 consecutive days. Subjects evaluated their itch symptoms, number of wheals, and size of the largest wheal twice daily, and the effect of their skin condition on the quality of sleep and normal daily activity. At each visit, the investigator assessed the number of wheals, size of the largest wheal, erythema, and skin area involved. At the final visit, the subject and investigator rated their perception of the treatment effectiveness. Safety and tolerability were evaluated.
Results: Compared to placebo, vapitadine significantly improved the itch severity, reflected in the mean subject's daily assessments of itch score ($p = 0.038$). Subject's assessments of other urticaria symptoms as well as QoL parameters showed a nonsignificant trend in favour of vapitadine. The subjects as well as the investigators gave vapitadine treatment a significantly better global assessment score than the placebo treatment ($p = 0.020$ and 0.013, respectively). Overall, there were no safety issues in this trial. The incidence of AEs was low. No clinically significant changes in vital signs and in clinical laboratory test results from screening to end of treatment were noted, as well as in post-treatment changes in ECG morphology.
Conclusion: Oral vapitadine, administered at a dose of 60 mg once daily for six days to subjects with CIU, significantly reduced the itch symptoms in comparison to placebo. Treatment was very well tolerated. No sedation or somnolence was reported. These results warrant further studies with vapitadine in this indication, to corroborate the findings in a larger population and to extend the treatment duration.

O6

H_3/H_4 receptor ligands modulate IgE activity induced by histamine

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It is well-known that histamine is a multifunctional mediator and mediates its effects through four pharmacologically distinct subtypes of receptors (H_1R - H_4R).
Through recent cloning of human histamine H_3 and H_4 receptors new studies have suggested that H_3/H_4 receptors may possess a wide range of pharmacological activities including effects on IgE synthesis in atopic and non-atopic diseases (Khanferyan R. *et al.* 2001-2008). The goal of the present investigation was to evaluate the involvement of H_3/H_4 receptors in IgE-modulatory activity of histamine. Several IgE levels were assayed for total IgE by CAP FEIA method (Phadia) in supernatants of 9-day cultures of the PBMC of 10 healthy subjects and 12 ragweed sensitive patients. The latest PBMC were cultivated during remission and in acute clinical stage (high pollen season time - august-september). PBMC were cultivated with and without histamine (10^{-8}M and 10^{-5}M). Imidazole and non-imidazole antagonists having varying affinity and potency (FUB 181, FUB 649, FUB 372, Imoproxifan – IMP) were studied as H_3/H_4 receptor antagonists. In the preliminary study, it has been shown that histamine effects on IgE synthesis in healthy donors depends on the concentration of agonist. IgE synthesis suppressed under the high concentration (10^{-5}M) and was stimulated by low concentration (10^{-8}M) of histamine. The effects of H_3/H_4 receptor antagonists on IgE-modulatory activity of histamine highly differ in healthy volunteers and allergic subjects. Thus, all studied H_3/H_4 receptor antagonists in healthy donors diminished IgE-supressory activity of the high concentration of histamine. In remission, the high affinity antagonist (Ki = 0.26 nM) IMP showed most pronounced effect on histamine induced IgE synthesis. At the same time, all studied H_3/H_4 receptor antagonists together with histamine (10^{-5}M) developed a co-suppressory effect on the IgE synthesis in PBMC of patients cultured during the pollen season. Thus, the spontaneous IgE synthesis, as well as histamine-induced IgE synthesis, depends on the affinity/potency of H_3/H_4 receptor antagonists and may differ in healthy donors and allergic patients in different clinical stages.

A knowledgement: We would like to thank Prof. W. Schunak providing us H_3/H_4 receptor ligands for this investigation.

O7

The Histamine H4 receptor (H4R) Mediates Inflammation and Pruritus in Th2-dependent Dermal Inflammation

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The histamine H4 receptor (H4R) has previously been shown to mediate allergic inflammatory and pruritic responses. In this study the role of H4R was investigated in a Th2 cell-mediated mouse skin inflammation model that mimics several of the features of atopic dermatitis. Treatment with two specific H4R antagonists before challenge with fluorescein isothiocyanate (FITC) led to a significant reduction in ear edema. This was accompanied by a reduction in the levels of several cytokines and chemokines in the ear tissue. Upon ex vivo antigen stimulation of lymph nodes, H4R antagonism reduced the antigen-induced lymphocyte proliferation and the levels of IL-4, IL-5 and IL-17. One explanation for this finding is that lymph nodes from animals dosed with the H4R antagonist, JNJ 7777120, contained a lower number of FITC positive dendritic cells. The effect of H4R antagonism on dendritic cell migration in vivo may be an indirect result of the reduction in tissue cytokines and chemokines or a direct effect on chemotaxis. In support of a direct effect, dendritic cells treated with JNJ 7777120 were defective in migration towards CCL21 in vitro. In addition to anti-inflammatory effects, JNJ 7777120 also significantly inhibited the pruritus exhibited in the model. Therefore, the dual effects of H4R antagonists on pruritus and Th2 cell mediated inflammation point to their therapeutic potential for the treatment of Th2-mediated skin disorders including atopic dermatitis.

O8

Histamine oxidation is mainly controlled by AOC3 amine oxidase in mouse adipose tissue

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Adipose tissue (AT) development can be modified by alterations of the histaminergic status: histamine-deficient mice exhibit visceral obesity (Fülöp *et al.,* Endocrinology, 2003;144:4306-14) and prolonged treatments with H_3-antagonists reduce body weight gain and adiposity (Hancock & Brune. Expert Opin Investig Drugs 2005;14:223-41). Direct H_2-histaminergic stimulation of lipolysis has been described in canine fat cells, but histamine effects on AT remain poorly documented in murine and human fat cells. It has only been observed that histamine can stimulate lipolysis in rodent adipocytes when its oxidation by amine oxidases is blocked by pharmacological agents like semicarbazide. The aim of this work was to study the influence of AOC3 gene invalidation (encoding for semicarbazide-sensitive amine oxidase, SSAO) on histamine oxidation in peripheral tissues, on histamine lipolytic activity in adipocytes, and on adiposity. In wild-type mice, histamine oxidation was lower than that of the reference SSAO-substrate benzylamine, in AT and kidney, but not in the ileum. The relative order of magnitude regarding hydrogen peroxide formation during 1 mM amine oxidation was: AT > ileum >> kidney. In AOC3-KO mice, there was a complete abolition of benzylamine oxidation in all the tissues, while histamine oxidation was abolished in AT but not in ileum. This, together with the expression levels of the genes encoding for AOC1, AOC2 and AOC3, indicated that histamine was mainly oxidized by diamine oxidase (DAO, the AOC2 product) in intestine, but by SSAO (the AOC3 product) in AT. Histamine, which was unable to activate lipolysis in subcutaneous adipocytes from wild-type mice, significantly stimulated lipolysis in fat cells from AOC3-KO mice. However, histaminergic lipolytic activity did not reach the maximal intensity of beta-adrenergic stimulation. All together, these data indicate that, when protected from the oxidation it undergoes by SSAO in AT, histamine is able to directly activate lipolysis in isolated mouse adipocytes. However, such *ex vivo* histaminergic mobilization of lipids does not seem to occur spontaneously *in vivo* since basal lipolytic activity is reduced in the AOC3-KO mice which do not have less fat mass than their wild type controls. Whether histamine tissue content is altered or whether other factors regulating adiposity are modified in AOC3-KO mice remains to be established.

O9

Spinal Histamine H_4 receptor expression in a rat chronic inflammatory pain model

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We have previously shown that the histamine H_4 receptor is functionally expressed both on immune and neuronal cells, which offers the intriguing possibility that it may represent a dual target for chronic inflammatory pain therapies (Dijkstra *et al.,* 2007; Connolly et al., 2009). We have reported recent evidence for plastic changes in both the spinal and supra-spinal nociceptive pathway in a chronic inflammatory pain model, which seeks to closely mimic persistent responses seen in the clinic, by introducing the inflammatory agent Complete Freunds Adjuvant (CFA) into the knee joint and monitoring hypersensitivity for up to 90 days (Wilson *et al*., 2005; Chazot *et al*., 2008). We hypothesise that changes in histamine H_4 receptor may contribute to the chronic hypersensitivity in these animals. For this present study, rats were sacrificed 16 days post-CFA and spinal cords (n=8 sham, n=8 CFA) dissected and processed. Semi-quantitative immunohistochemical analyses were performed using two validated anti-H_4R antibodies (Dijkstra *et al.,* 2007, Lethbridge & Chazot, unpublished), initially to define the anatomical profile of the H_4R in the spinal cord and secondly to assess any potential changes in expression following CFA treatment. Anti-H_4R immunoreactivity was detected in the dorsal horn of the rat spinal cord at both the lumber and thoracic level in laminae I and II. This immunoreactivity was greatly suppressed by prior incubation with the respective peptide immunogens. Preliminary analysis (n = 8 replicate sections from 2 independent CFA- and sham-treated animals), indicated a reduction in anti-H_4R immunoreactivity intensity in Laminae I and II at the thoracic level of the CFA-treated compared to sham-treated rats. This requires further replication to confirm these initial findings.

Chazot PL *et al.* Acta Physiologica Sinica (Suppl.) 2008; P1-03-07; Connolly *et al* Br J Phamracol. (2009) (in press); Dijkstra D *et al.* Journal of Allergy and Clinical Immunology 2007; 120:300-307; Wilson AW *et al.* Eur Journal of Pain 2005;
10: 53749
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O10

Histamine H3 receptor antagonists with antipsychotic components

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Schizophrenia is a psychiatric disease that is linked with the dysregulation of dopaminergic and other neurotransmitter systems, mainly in cortical and subcortical brain regions. These participate in cognitive, emotional and motivational behaviour that is disordered in schizophrenic patients and appears in form of positive and negative psychotic symptoms. Modern therapy focuses on typical and atypical neuroleptics, which mainly act as modulators of aminergic neurotransmitters in the central nervous system (CNS).[1]

Human histamine H_3 receptors ($h$$H_3$R) in the CNS function as auto- and heteroreceptors, modulating synthesis and release of neuronal histamine as well as liberation of various other neurotransmitters. Histaminergic neurons and their projections are co-localised to distinct brain areas associated with the development of schizophrenia. Inverse agonists/antagonists of $h$$H_3$R, including tiprolisant, ABT-239, or GSK-189254, increase dopamine concentration in rat prefrontal cortex and show some antipsychotic properties in preclinical and clinical studies.[2]

The aim of this study, which is the development of novel highly affine $h$$H_3$R antagonists with an optimised target profile for potential therapeutic purpose, has been realised by coupling the common 4-(3-piperidinopropoxy)phenyl $h$$H_3$R antagonist pharmacophore to typical and atypical antipsychotic drugs like amitryptiline, maprotiline, chlorpromazine, chlorprothixene, fluphenazine, and clozapine. Novel compounds are tested *in vitro* for affinity at some aminergic G protein-coupled receptor subtypes. Most of them exhibit excellent $h$$H_3$R affinities in the (sub)nanomolar concentration range, mainly maintain $h$$D_2$R/$h$$D_3$R affinities and show reduced affinities at $h$$H_1$R, a non-target. These newly profiled multi-targeting compounds may display an optimised antipsychotic outline in their potential therapeutic properties.[3]

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O11

Selective H_4R antagonist prevents antigen-induced airway inflammation in guinea pig: a pivotal role of annexin-A1

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Although mechanism involved in the pathogenesis of asthma remain unclear, role for oxidative/nitrosative stress have been documented. Recent evidences suggest that histamine has a key role in allergic inflammation through the activation of histamine H_4 receptor, a novel G-protein coupled receptor. Annexin-A1 (lipocortin-1, LC-1), a 37 kDA anti-inflammatory protein that inhibits the activity of cytosolic phospholipase A_2 ($cPLA_2$), plays a key role in the production of lipid inflammatory mediators such as prostaglandings (PGs) and leukotrienes (LTs).
Here we report the effects of compund JNJ 7777120 (JNJ), a selective H_4 receptor antagonist, on antigen-induced airway inflammation and LC-1 levels in brochoalveoar lavage (BAL) fluid.
Ovalbumin-sensitized guinea pigs placed in a respiratory chamber were challenged with the antigen. JNJ, at the dose of 5, 7.5 and 10 mg/Kg b.wt. was given i.p. for 4 days before OA challenge. Respiratory parameters were recorded and quantified.
BAL fluid was collected and the lung removed from guinea pigs 48h after OA challenge. In the BAL fluid, the levels of LC-1, PgD2, LTB4 and TNFα were determined. Myeloperoxidase and caspase-3 activities, 8-hydroxy-2-deoxyguanosine and MnSOD were evaluated in lung samples.
OA-challenge decreased significantly LC-1 levels in BAL fluid, associated with respiratory abnormalities and a significantly increase of PgD_2, LTB_4, and TNF-α levels. Treatments with JNJ significantly and dose-dependently increase the levels of LC-1, reduced cough, dyspnea and severe bronchoconstriction and the levels of PgD_2, LTB_4, and TNF-α in BAL fluid. Moreover, inverse correlations among LC-1 and prostanoid and cytokine levels in BAL fluid were observed.
These results suggest that antigen-induced asthma-like reaction decreases the levels of LC-1 with a consequent increase in TNF-α and eicosanoids production. JNJ pre-treatment modulate allergic asthmatic response and airway inflammation throughout an up-regulation of LC-1.

O12

Histamine H4 receptor antagonists A-943931 and A-987306: in vitro profiles, and efficacy in pain, inflammation, and itch models

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A novel class of histamine H_4 receptor antagonists was designed by introducing rotational constraints into an aminopyrimidine lead series, and optimizing the new series for potency and selectivity in binding and functional assays. A-943931 and A-987306 in particular demonstrated potent (nM) antagonism of human, rat, and mouse receptors, as assessed in functional assays in vitro (blockade of histamine-induced FLIPR Ca^{2+} flux and ^{35}S-GTP binding in H_4-transfected cells, and blockade of chemotaxis in bone marrow derived mast cells) and *in vivo* (blockade of H_4 agonist-induced scratching in mice). The compounds blocked zymosan-induced peritonitis in mice, as expected for H_4 antagonists. Members of the new series were profiled and found to have good PK properties in rats and mice ($t_{1/2}$ ranged 1.6-4.9 hours, and oral bioavailability ranged 26-90%). The new series was able to efficiently permeate CNS tissues (with brain/plasma ratios of 2 to 6), a property of potential significance in light of recent reports of expression of H_4 receptor in the CNS. Most surprising was the finding of efficacy of the compounds in pain models, including inflammatory (carrageenan) and neuropathic (Chung spinal nerve ligation) pain models. Substantial evidence has accumulated supporting the potential of H_4 antagonists for treatment of inflammatory disorders, such as asthma and colitis. A-943931 and A-987306 reinforce this, as well providing support for new findings of the potential utility of H_4 antagonists to treat different types of pain. These compounds should serve as useful tool compounds to continue to expand the understanding of role of H_4 receptors in disease.

O13

Histamine treated rats with occlusion of gastric artery and pylorus ligation develop gastric lesions progressing into chronic ulcerations. Gastroprotective effect of prostaglandins

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Background and Aims: Histamine is not only a potent stimulator of gastric acid secretion but also plays a central role in gastroduodenal ulcerogenesis. In the present study, we tested the effect of pre-treatment with exogenous prostaglandin E_2 (PGE_2) in a new model of experimental gastric ulcers induced by combination of histamine and gastric ischemia in rats. Methods: In 40 Wistar rats a chronic ischemia of gastric mucosa was induced via clamping of left gastric artery and vein (L-AV) and pylorus ligation for 4h and 8h. Following treatment, groups of rats were investigated: 1) histamine alone (40µg/kg twice s.c.); 2) vehicle (saline) followed 30 min later by gastric mucosal L-AV ischemia and pylorus ligation combined with histamine (40 µg/kg twice s.c.) and 3) PGE_2 (5µg/kg i.g.) followed 30 min later by gastric mucosal L-AV ischemia combined with histamine (40 µg/kg twice s.c.) and pylorus ligation. At 4 and 8 hrs upon the clamping of L-AV and pylorus ligation, the area of gastric lesions and the gastric blood flow (GBF) was determined by H_2-gas clearance, the mucosal expression and plasma of proinflammatory cytokines, TNF-alpha and IL1beta,were assessed by RT-PCR and ELISA, respectively. Results: Histamine treatment failed to produce gastric lesions but when it was combined with ischemia, the widespread gastric lesions in the corpus mucosa, but not in antrum, were observed. This ulcerogenic effect was accompanied by the significant fall in the GBF, the overexpression of IL-1beta and TNF-alpha mRNA in the gastric mucosa and the significant increase in their plasma levels. This damaging effect and decrease in the GBF were significantly attenuated by pretreatment with PGE_2. Both, the strong upregulation of mRNA for proinflammatory cytokines TNF-alpha and IL-1beta and their plasma levels were also significantly diminished in rats pretreated with PGE_2. Conclusion: The present study demonstrates that gastric hypersecretion induced by histamine in combination with gastric mucosal ischemia results in gastric lesions which progress into chronic gastric ulcers and can be used for the screening of new anti-ulcer therapy.

O14

Gut mediated pseudoallergic reactions in atopic and environmental patients

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Atopic and environmental diseases are commonly associated with an increased environmental load of exogenic and endogenic origin, whereby chronic intestinal toxicosis is one of the major factors involved.
Quantitative investigations of the intestinal flora in duodenal aspirates and stool samples are consistently showing a significant reduction of physiological lactic acid producing flora (lactobacilli, bifidobacteria, enterococci) associated with increased colony numbers of facultative pathogenic strains such as *haem. E. coli, Proteus, Klebsiella, endotoxigenic clostridia,* Bacteroides and yeasts/ molds including *Candida, Geotrium, Aspergillus, Penicillum, Mucor*, respectively. Their fermentation and putrefaction by-products including alcohols, aldehydes, CH4, H_2S, indole, phenol, cresol, tartrate, biogenic amines, endo- and mycotoxins are maintaining an increased gut permeability, high histamine release, inflammation and liver toxic burden. This allows in turn a high antigen entry with subsequent immune sensitisation, generation of high levels of circulating immune complexes with activation of platelet aggregation and exhausting of liver RES and detox functions.
Consequently, atopic and environmental patients are often depicting disturbed Phase I and/or Phase II detox activities as recorded by means of genetic polymorphism analysis or liver detox texts.
Major therapeutic interventions may include specific antimicrobial compounds followed by long term pre- and probiotic substitution, the administration of toxin absorbers, diet changes and appropriate supporting measures for the detox phases I and II (GSH, polyphenols, glucuronic acid precursors, cruciferous reach food, PUFAs)

Reference:
Ionescu J.G.: Allergens, Infections and Environmental Pollutants involved in the Pathogenesis of atopic eczema. Russian Allergology Journal 4, 11-16, 2004.

O15

Differences in activation patterns between peripheral blood and cord blood derived human mast cells

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Human mast cells (MC) can be cultured *in vitro* using numerous media and several kinds of progenitors from different sources. However, it is unclear how different protocols for *in vitro* derived human MC affect the final phenotype.
The purpose of this study is to investigate the activation pattern of human MC derived from different progenitor sources, but cultured under the same conditions.
CD133+ cells isolated from either buffy coat blood or fresh heparinised cord blood using a CD133 purification kit (MACS) and cultured in Stem Span containing 50 ng/ml IL-6, 100 ng/ml SCF and 100 g/ml Penicillin/Streptomycin. In addition, cultures were given 1 ng/ml IL-3 for the first 3 weeks and 15% FCS at week 6, and mature cells were used at week 7.
MC sensitized with IgE were incubated with either Compound 48/80, Substance P, Codeine Phosphate or anti-IgE for 1 hour. Supernatants were analysed for histamine, using a fluorescence method (RefLab Aps) and expressed as % histamine release (HR) using a whole cell lysate as the total histamine level.
Using 5 mg/ml Compound 48/80 no difference was found in HR between peripheral blood derived MC (PBdMC, n=6) and those derived from cord blood (CBdMC, n=8) (34% vs 38% HR). However, using 10 or 100 times less Compound 48/80 PBdMC reacted significantly stronger (22 % vs. 10 % for CBdMC).
Only PBdMC could be activated by Substance P (1, 4 or 10 mM) with a HR ranging from 17 to 25% and Codeine Phosphate (0.5 or 0.1 mg/ml) with a HR of 25%. Furthermore, anti-IgE stimulation (80 - 625 ng/ml) resulted in a significantly higher HR in PBdMC (ranging from 15 - 25%) with response of CBdMC on detection limit.
Human MC derived from different progenitor sources but cultured under the same conditions do not have the same reaction patterns when stimulated with known MC activators. Unique phenotypes of MC might be obtained from individual protocols and it is therefore important to characterize in vitro derived MC in order to compare the results.

O16

Human lung mast cell 'releasability': role of syk

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Previous studies in a variety of mast cell systems have shown that the protein tyrosine kinase, syk, plays a critical role in transducing FceRI-mediated signals. In human basophils, 'releasability' has been linked to the extent of syk expression (MacGlashan, 2007). Human lung mast cells, like basophils, are also found to be variably responsive to IgE-dependent activation. The principal aim of the present study was to determine whether the wide variability in human lung mast cell responses, following IgE-dependent activation, is linked to syk expression.

Mast cells were isolated by physical and enzymatic disruption of human lung tissue. Cells were further purified by flotation over Percoll gradients and immunomagnetic bead separations. 'Releasability' was determined by activating the cells with a maximal releasing concentration of anti-IgE (1:300) and measuring the extent of histamine release. Syk content in mast cells was determined by immunoblotting and flow cytometry.

In this study (n=600) we found that histamine release from mast cells challenged with a maximal releasing concentration of anti-IgE ranged from 0 to 74% (mean 24±1%) and 13% of these preparations were 'non-releasers'. Immunoblotting studies indicated that, compared to mononuclear cells, human lung mast cells express low and quite variable levels of syk. However, there was no correlation between syk expression and mast cell releasability (n=30). This finding was supported by studies where no correlation was observed between releasability and syk content as determined by flow cytometry (n=9). Nonetheless, a number of putative inhibitors of syk including NVP-QAB205 (EC_{50}, 0.2 μM) effectively attenuated the IgE-dependent release of histamine from mast cells (n≥5).

These studies indicate that, although syk may play an important role in mediating degranulation, syk does not appear to govern mast cell releasability.

MacGlashan DW (2007). *J Allergy Clin Immunol* **119**: 626-633.

O17

Histamine Prevents Functional and Histological Alterations of Salivary Glands Exerted by Ionizing Radiation

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Xerostomia is a common, disturbing side effect among patients treated with radiotherapy for head-and-neck cancer leading to considerable morbidity. It is associated with severe functional and structural alterations of salivary glands causing secretory hypofunction. The submandibular gland (SMG) is one of the major salivary glands, being the main producer of saliva.

We have previously demonstrated that histamine treatment significantly protects small intestine and bone marrow against radiation-induced toxicity.

The aim of the present study was to investigate whether histamine could prevent salivary gland dysfunction and histology alteration exerted by ionizing radiation.

For that purpose, 30 rats were divided into 4 groups. Histamine and Histamine-10Gy groups received a daily histamine injection (0.1 mg/kg *sc*) starting 20 hours before irradiation. Histamine-10Gy and untreated-10Gy groups were irradiated with a single dose on whole-body using Cesium-137 source. 3 days post-irradiation metacholine induced salivary secretion was measured or animals were sacrificed and SMG removed, fixed, stained with hematoxylin and eosin and histological characteristics were evaluated. We studied, by immunohistochemistry, the expression of proliferation markers, histidine decarboxylase (HDC) and histamine content.

Results indicate that radiation decreased salivary secretion by 40% in comparison to untreated rats, which was associated with a loss of SMG mass and an alteration of epithelial architecture, partial loss of secretor granular material, anisokaryosis and diminished proliferation. In contrast, histamine completely reversed the reduced salivation induced by radiation, significantly conserved glandular mass with normal appearance and preserved structure organization of secretor granules. Histamine and HDC was detected principally in ductal cells.

We conclude that histamine prevents radiation-induced damage on SMG being of potential clinical value for patients undergoing radiotherapy.

O18

Effect of some lipoic acid derivatives and other anti-oxidants on the histamine-induced contraction of guinea-pig tracheal smooth muscle

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The intracellular milieu is kept in a reduced state through the delicate balance between oxidants and antioxidants, and a high content of free protein thiols. Thiols have been shown to inhibit mast cell (MC) and basophil leucocyte activation, airway (tracheal) smooth muscle (TSM) contraction [1], as well as airway inflammation and hyper-responsiveness in a mouse asthma model [2], all suggesting the potential value of antioxidants in airway inflammation and asthma. To that end, we have studied the modulation of guinea-pig TSM contraction by some antioxidants: lipoic acid (a natural disufide antioxidant) and two newly synthesised derivatives, UCLM084 (a benzamide) and UCLM109, a hybrid of lipoic acid with the histone deacetylase inhibitor MGCD0103 (which inhibits MC activation and TSM contraction by antigen and TSM agonists)[3], glutathione and sodium ascorbate.
All the antioxidants had a relaxant effect on resting tension and on tension induced by histamine or carbachol, EC_{50}(s) of 0.2-5.0 mM. Pre-incubation of TSM with antioxidants for 30 min had a dual effect on responsiveness to exogenous histamine: potentiation at a relatively low antioxidant concentration (<500 μM), and inhibition at millimolar concentrations of the antioxidant. By contrast, the antioxidants had a 'single' action on TSM contraction by carbachol or 5-hydroxytryptamine (5-HT): a concentration-dependent inhibition. Furthermore, they more potently blocked the contraction by carbachol and 5-HT than that by histamine. The potentiation of histamine-induced TSM contraction by low antioxidant concentrations varied from one antioxidant to another, e.g. Na ascorbate at < 500 μM caused a small potentiation of histamine, while ascorbic acid produced a more marked potentiation. The potentiation of histamine-induced contraction of rabbit aorta by ascorbate at 5-500 μM has been reported [4]. The underlying mechanism of these effects remains to be elucidated, including whether nitric oxide formation is involved.

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O19

Inhibition of histamine release and intracellular protein synthesis by histamine high dilutions

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Background: In this such debated field of the putative biological effect of high dilutions on basophil activation, the majority of the results published so far were obtained from the analysis of membrane markers immunomodulation by serial dilutions of histamine. Our objective was to study, on the mouse bone marrow leukocytes model, the effect of these dilutions on histamine and IL4 release.
Objectives: Our objective was to study, on the mouse bone marrow leukocytes model, the effect of these dilutions on histamine and IL4 release, in the same technical conditions than for the human basophil model
Methods: Histamine dilutions were prepared in polystyrene 20 ml tubes from a 10^{-1}M mother dilution of histamine hydrochloride up to the 18^{th} dilution (dilution 18C) with a dilution ratio of 100. These dilutions were kept at +4 C before use with a maximum storage delay of 1 month. Histamine and IL4 production were measured after anti-IgE and IL3 induced activation, histamine being measured by the automatized fluorimetric technic and IL4 by ELISA. We compared also, in the same conditions the reactivity of bone marrow cells freshly prepared (A) and after 8 days of culture in the presence of IL3 (B).
Results: Histamine high dilution 15CH inhibited both histamine production induced by IgE (3.5%, p=0,042, n=10 (A) and 4.1%, p=0,016, n=5 (B)) and by IL3 (8.4%, p<0,001, n=14 (A) and 7.6%, p=0,021, n=6 (B). The same histamine dilution inhibited also IL4 production induced by IL3 but only on cultured cells (34%, p=0,007, n=6). The effect of histamine 15 CH dilution was not significant on IL4 production induced by IL3on fresh cells and on IL4 production induced by IgE on the two types of cell suspensions. Results obtained in parallel with the histamine high dilution 15 CH were not significant.
Conclusions: These results represent a confirmation of the biological effect of histamine high dilutions. In this mouse model histamine 15 CH dilution was capable of inhibiting the production of the two major basophil mediators histamine and IL4. The effect on IL4 production (34% inhibition) showed that high dilutions may interfere with intracellular protein synthesis.

O20

Reliable rapid detection of allergen-specific and total IgE in the diagnosis of type I hypersensitivity using a novel allergy lateral flow assay (ALFA)

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Type I allergies are characterized by the involvement of allergen specific IgE, thus the detection of specific IgE is an important part of modern allergy diagnostics. State-of-the-art allergy diagnosis includes detailed patient's case history, physical examination, skin prick testing and *in-vitro* tests for the detection of IgE. Furthermore, provocation challenges and / or cellular tests such as the basophil degranulation test are needed especially in case of food allergies. Today a high number of commercial test systems are available for sIgE and total IgE detection. However, just recently, rapid assays based on the lateral flow assay technology became available for the detection of specific IgE such as ALFA (Allergy Lateral Flow Assay). ALFA is based on a universal test device to use with various allergen solutions. This simple and user-friendly test system offers the opportunity for patients to test themselves for specific IgE to various allergens within 20 minutes as a first line screening test. In addition, ALFA can also be used by general practitioners in primary care as a screening test using allergen mixtures or single major allergens.
Comparative studies have been performed showing good agreement between ALFA and the skin prick test and laboratory methods for the detection of specific IgE. Therefore, rapid assays for the detection of specific IgE, especially ALFA, represent promising tools for the early diagnosis of type I allergies. This presentation introduces the principle of ALFA, and summarizes all important studies on ALFA compared to assays based on the classical protocol for the detection of specific IgE.

O21

The Function of Transcription Factor E2A in CD8 Cell Regulation

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Paradoxically, autoimmune disease and immune deficiency, which are usually regarded as two opposite extremes of immune response, can coexist in one patient. This paradox can be resolved by the homeostatic proliferation associated with lymphopenia. Homeostatic proliferation is best illustrated by the spontaneous proliferation and expansion of naive T cells after transfer into a T-cell deficient host. With less intensity, homeostatic proliferation is also observed in newborns as a physiological response. Compared to other mechanisms of T cell proliferation, homeostatic proliferation has unique features (stimulation by low affinity self antigens; adoption of a memory T cell - profile) which could facilitate destructive autoimmunity. The current model suggests that homeostatic proliferation primes naive T cells of broad specificities, possibly including otherwise quiescent autoreactive T cells, and that it overcomes T cell tolerance. In our study we examine the function of the transcription factor E2A, which is well known to be a key player in the early B and T cell development, in regulating homeostatic proliferation. Mature naive OT1-transgenic CD8 cells of wild type-, E2A-deficient- or E2A/GFP-mice are transferred into normal and irradiated lymphopenic hosts. Proliferation profiles (by CFSE labeling) and E2A-levels of the donor cells are monitored. It could be observed that donor cells in a lymphopenic environment have higher E2A levels compared to normal hosts and that E2A-deficient donor cells are prone to proliferation compared to wild type cells. E2A is a gatekeeper in the selection checkpoints during T cell maturation by suppressing premature proliferation and expansion of T cells. The evidence of E2A as a critical factor for the limitation of naive T cell proliferation after a lymphopenia-inducing event would open new avenues in the understanding of the critical balance in constructive versus destructive immune cell development and expansion.

O22

Histamine influence on apoptosis in trophoblast cell cultures.

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It has been demonstrated that histamine plays an important role in the pathogenesis of preeclampsia. Histamine regulates the process of differentiation of trophoblast cells; it also acts as a growth factor in malignant melanoma cells and prevents monocytic apoptosis. Trophoblast research has shown that in pre-eclampsia placentas, trophoblast apoptosis is significantly increased.

The aim of our study was to demonstrate the influence of histamine on the process of apoptosis in human trophoblast cell cultures.

Placentas were obtained after vaginal delivery. Tissue samples were excised from placentas and, with the use of modified Kliman's method, trophoblast cell cultures were established. The cultures were incubated with dexamethasone, as apoptosis inducer, 48 hours prior to apoptosis detection assays. Along with dexamethasone, selected cell cultures were incubated with histamine (1 µmol/l) or histamine (1 µmol/l) and terfenadine - H_1 receptor antagonist - (from 1 to 5 µmol/l). For apoptotic activity detection, and quantitative analysis, we used an ELISA assays. M30-Apoptosense ELISA Kit is based on the M30 monoclonal antibody that binds only the caspase-cleaved cytokeratin 18 formed during apoptosis in trophoblast cells.

Our investigation showed significantly ($p<0.05$) increased apoptotic activity in cultures incubated with dexamethasone, histamine and terfenadine (% of reference value, +/-SEM): up to 113.1% +/-4.33. Cell cultures incubated with dexamethasone and histamine only, showed significantly lower apoptotic activity 90.2% +/-5.17.

We suggest that histamine may inhibit apoptotic activity in trophoblast cell cultures via the H_1 receptor. Thus, histamine may regulate the process of trophoblast differentiation (via integrin aV-b3 expression, as we previously suggested), and influence cell turnover in placenta.

O23

The clinical usefulness of basophil histamine release

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Human basophils have, for half a century, served as an *in vitro* model of IgE-mediated reactions where the basophil cell function has been studied by measuring histamine release. Determination of released histamine has improved especially with respect to capacity and it is now possible to perform 400 single determinations on 10 mL heparinised blood. This high capacity method (HR-Test) is based on specific adsorption of histamine to a glass fibre coated matrix on microtiter plates and subsequent fluorometric determination of glass fibre bound histamine. The functional testing of basophils has been used extensively to study IgE-mediated inhalant, food, drug and occupational reactions. Thus, the HR-Test can be used for diagnostic purposes and to study the effect of immunotherapy which results in a down-regulation of basophil cell function and the development of neutralising/blocking antibodies.
In another allergy-like disease, chronic urticaria (CU), the basophils have attracted much attention. In this disease, autoantibodies against IgE/IgE-receptor have been identified and they can be detected by serum induced histamine release. Further, the number of blood basophils in CU patients is reduced and their basophils are functionally impaired with respect to IgE mediated histamine release whereas the cells are hyper responsive to their own serum
Probably the basophil is involved in other immunological non-IgE mediated reactions. Recent data from our laboratory have thus demonstrated that basophils is activated by incubation with T-cell receptor activated T-cells. Further, that activated basophils up-regulate and release TNFα, which is a key cytokine in infectious and autoimmune diseases, and this release induce MMP9 up regulation and release from monocytes. MMP is an important inflammatory metalloproteinase. Future studies will reveal the involvement of the basophil in non-IgE mediated innate and TH1 immunity.

O24

New molecular insights in the H4R-receptor field

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The human histamine H4-receptor (H4R) is postulated to play a role in a variety of conditions including atopic asthma, itch and the modulation of pain .1,2 Using a chemical genomics approach and a fragment based drug discovery effort we have identified a variety of new ligands for the histamine H4R receptor, including the H4R agonists VUF8430 and 4-methylhistamine. In the present talk, I will highlight our recent efforts to understand in detail the difference in binding profile of the histamine H3R and H4R receptor. Moreover, we have previously identified a pronounced species difference for a variety of compounds. Using site-directed mutagenesis and computational modeling we have not gained detailed insights in the various receptor-ligand interactions. These data on one hand explain the observed differences, but are also useful in the design of new H4R ligands.

1 Oda, T., et al. *J. Biol. Chem.* **2000**, 275:36781-36786
2 Lim, H., et al. *Curr. Top. Med. Chem.* **2006**, 6:1365-1373

O25

H4R-ligands – clinical aspects

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The discovery of a fourth histamine receptor (H4), and the realization of its exclusive expression on the hematopoietic cell types which are most implicated in the development and symptomatology of allergy and asthma, suggests that pharmacological targeting of the H4 receptor, either alone or in combination with H1 receptor antagonists, may prove useful for treating both allergy and asthma. In addition preclinical evidence is accumulating for a number of other indications including colitis, pruritus and, most recently, pain. This presentation will review the preclinical evidence that supports the clinical evaluation of H4R antagonist in these indications.

O26

Development of diaminopyrimidines as histamine H_4 receptor ligands – virtual screening and Scaffold optimisation

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The recently discovered human histamine H_4 receptor (*h*H_4R) is predominantly expressed peripherally on hematopoietic mononuclear cells. The *h*H_4R is involved in immunological and inflammatory responses because histamine signalling induces cell shape change and chemotaxis of mast cells and eosinophils, mast cell migration, as well as upregulation of adhesion molecules on monocytes. From these physiological reactions, one can deduce several potential indications for H_4R antagonists in the broad field of anti-inflammatory therapy.[1] With an aim to develop new H_4R antagonists a virtual screening[2] of SPECS database was performed. Based on the first reported selective H_4R antagonist JNJ-7777120, it resulted in two hits that contain triazolo- or thienopyrimidine scaffolds coupled to methylpiperazine. In competition binding assays, they exhibit affinities in the low micromolar concentration range. Degradation of the annelated aromatic core by fragmentation into its heteromonocyclic components showed that 2,4-diaminopyrimidine is a potent *h*H_4R affinity scaffold. This scaffold was further developed to the lead structure N^4-benzyl-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine, which has very recently also been approached by others. In first line, optimisation was carried out with consideration of *h*H_4R affinity: modification of the benzylamine moiety by spacer variation, by diversification of the substitution pattern according to classical Topliss scheme[3], and by rigidification, led to *h*H_4R ligands with affinities in the low nanomolar concentration range. Structure-activity relationships based on this scaffold reveal that slight structural changes evoke extensive differences, not only in potencies, but surprisingly also in functionality: while *o*-substituted benzylamines show partial agonism, *m*-substituted and rigidified congeners exhibit inverse agonist efficacy.

[1] Zhang M, Thurmond RL, Dunford PJ. Pharmacol Ther 2007; 113:594-606.
[2] Schneider G, Baringhaus KH. Weinheim, New York 2008.
[3] Topliss JG. J Med Chem 1972; 15:1006-1011.

O27

Severity of seizures and neuronal damage are enhanced in the 9-day-old histamine 1 receptor knockout mice

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The functional disturbance of the central histaminergic neurons is proposed to contribute to the severity of epilepsy. Our recent study indicated that the tuberomamillary histaminergic neurons protect the hippocampus from kainic acid (KA)-induced neuronal damage through H_1 receptors in the organotypic coculture system (Kukko-Lukjanov et al. 2006). We have now further examined the role of H_1 receptor-mediated regulation of KA-induced seizures and neuronal damage in the immature H_1 receptor knock out (KO) and age-matched wild-type (WT) mice.
The severity of the behavioural seizures was significantly enhanced in the 9-day-old (P9) H_1 receptor KO mice when compared to the WT mice at the KA dose of 2 mg/kg. The total duration of the behavioural seizures was significantly longer in the KO mice when compared to the WT mice both at the KA doses of 2 and 3 mg/kg. Moreover, neuronal damage was significantly enhanced in the CA1 region of the hippocampus, thalamus, and retrosplenial granular cortex (RGC) in the KO mouse when compared to the WT mice. Finally, the H_1 receptor antagonist, triprolidine (10 mg/kg) resulted in significantly more severe seizures when compared to the KA-treated WT mice. Triprolidine also significantly increased KA-induced neuronal damage in the septum, thalamus, and RGC when compared to the WT mice.
Our results indicate that H_1 receptor deficiency increases the severity and duration of seizures in the developing mice. In addition, more severe seizures resulted in enhanced neuronal damage in the specific brain regions of the H_1 receptor KO mice. Our results suggest that the H_1 receptor plays an important role in regulating the severity of seizures and seizure-induced neuronal damage in the immature mice.

Kukko-Lukjanov TK, Soini S, Taira T, Michelsen KA, Panula P, Holopainen IE.
J Neurosci. 2006; 26:1088-97.

O28

Effect of the H_4 Receptor Antagonist JNJ7777120 on Histamine Levels of Peripheral Blood Vessels in Rats with Adjuvant Arthritis

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Arthritis-associated inflammation may alter vascular responses that contribute to systemic manifestations of the disease. Besides its role in immune system (patho)physiology, histamine (HI) regulates vasculature responsiveness. The recently identified H_4 receptor has attracted wide interest due to its potential therapeutic exploitation in inflammatory disorders [1], yet its characterization in blood vessels is elusive. This study investigated the effects of the H_4 receptor antagonist JNJ7777120 on the HI content of large arteries and veins in a rat model of adjuvant arthritis. Male Wistar rats of 200-250g bw received complete Freund's adjuvant (CFA) and/or 30mg/kg JNJ7777120 at day 0 [2]. Following sacrifice at day 20, the abdominal aorta (AA) from below the renal arteries to its bifurcation into the iliac branches and the inferior vena cava (IVC) from its bifurcation in hepatic veins to below the diaphragm were dissected out. Tissue HI was quantified fluorometrically [2]. Differences between treatments and/or tissues were located by paired t-test, non-parametric statistical analyses and Anova. Lower HI levels were detected in the AA than in the IVC in arthritic rats. Administration of JNJ7777120 reduced IVC HI levels in adjuvant-treated animals. The results provide first evidence towards the H_4 receptor functionality in peripheral blood vessels. Interestingly, the JNJ7777120-induced reduction of HI levels in blood vessels, contrary to the failure of the antagonist to exert a related action on cartilage in this animal model [2] provides the lead for ongoing research on H_4 receptor systemic function that may prove beneficial in understanding the complex pathophysiology of the arthritic phenotype.

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O29

Murine and Human Langerhans Cells Express a Functional Histamine H_4 Receptor: Modulation of Cell Migration and Function

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Langerhans cells (LC) represent professional antigen presenting cells in the skin and, therefore, have an important role in the initiation and the maintenance of allergic skin diseases. However, it is not completely understood which mediators influence LC migration and function in these diseases. Histamine (HA) is a soluble mediator that is released at high concentrations in lesions of allergic skin diseases and numerous immunomodulatory functions of HA have already been described. The interaction between HA and LC, particularly via the newly described HA H_4 receptor (H_4R) has been scarcely investigated thus far. However, recent findings have demonstrated that other dendritic cells possess a functional H_4R.

Therefore, we investigated the expression and function of the H_4R on murine and human LC. The expression rate of the H_4R was evaluated by immunofluorescence staining in murine and human skin samples and by flow cytometry of epidermal single cell suspensions. The function of the H_4R was determined by intracellular flow cytometric measurement of chemokine production and LC migration assays.

We demonstrate that murine and human LC expressed the H_4R. The level of intracellular CCL2 production in human LC was reduced after stimulation with H_4R agonists and basal production could be restored when H_4R was blocked with the specific antagonist JNJ7777120. Moreover HA and a H_4R specific agonist augmented the migration of LC from the epidermis as shown in *ex vivo* migration experiments using human skin and *in vivo* migration experiments in mice.

In conclusion, the H_4R is expressed on murine and human LC and influences the immunomodulatory function and migration of these cells. These findings underline the relevance of the H_4R in allergic skin diseases and encourage further exploration of the H_4R as a therapeutic target in allergic skin diseases.

O30

Histamine H_3 receptors in Lewy Body dementias: an autoradiographical study with [^{3}H] GSK189254

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Dementia with Lewy Bodies (DLB) is a primary neurodegenerative dementia which shares clinical and pathological characteristics with both Alzheimer's Disease (AD) and Parkinson's Disease with Dementia (PDD). Patients suffer from a variable combination of dementia, mobility deficits and psychotic episodes. There is growing evidence for the involvement of the histamine H_3 receptor in attention and cognitive function, with H_3R antagonists displaying pro-cognitive effects in a range of pre-clinical animal models. The aim of the present study is to investigate the abundance of the H_3R in a range of cortical and striatal regions in human post-mortem brain, and to determine whether any changes occur in DLB, PDD and AD compared to normal age-matched control cases. Previous autoradiographical studies have employed radioligands which are now known to bind also with the histamine H_4R, which necessitates the need to re-evaluate such data in the light of this new information. Herein, we employed [^{3}H] GSK189254 (0.25nM), a new high affinity and selective H_3 receptor antagonist radioligand , and R-alphaMethylhistamine (10uM) to define non-specific binding, essentially as described in Medhurst *et al.* (2007). Coronal human brain sections (levels 9-15 Brodmann Areas) were employed (controls, mean age 78 ± 8; DLB, mean age 81 ± 6; PDD, mean age 76 ± 7; all agonal state 1, n = 12-14 for each cohort). Strong labelling was detected in insular, entorhinal and cingulate cortex, globus pallidus, caudate and putamen. Interestingly, preliminary analysis of the DLB/PDD (n=11 cases) and age-matched control (n=9 cases) showed no significant differences in H_3R levels in these brain structures, consistent with recent data with Alzheimer's disease cases (Medhurst *et al*., 2007). A more complete analysis of all cases examined is required to support these preliminary findings. This study further supports the potential of targetting the apparently preserved H_3R for therapeutic benefit in a wide range of human dementias.

Medhurst AD *et al.* J Pharmacol Exp Ther. 2007; 321:1032-45.

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O31

Mast Cell and Basophil Markers for Predicting Severity of Allergic Reactions to Food

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Mast cell and basophil degranulation is a cardinal feature of allergic reactions. Serum levels of mast cell and basophil products, including histamine and tryptase, have been shown to increase in some cases of anaphylaxis. Our objective has been to investigate whether baseline serum levels of these products may be useful in predicting those at risk of severe reactions to food.
Mast cell tryptase and carboxypeptidase levels were measured in serum obtained from 31 kiwifruit-allergic patients, with histories of severe systemic, mild systemic or isolated oral allergy, using specific ELISA techniques developed within our laboratory. Levels were also measured in serum from 15 control subjects. PAF is thought to be released from mast cells and basophils, and the enzyme that degrades it, PAF acetylhydrolase, has recently been implicated in anaphylaxis (Vadas *et al. NEJM*.2008;**358**:28-35). We have determined activity of this enzyme in the serum samples by measuring hydrolysis of 2-thioPAF. Low serum ACE levels have been linked to pharyngeal edema in reactions to nuts (Summers *et al*. *JACI*.2008;**121**:632-638). Serum levels of ACE have been measured in this study by ELISA. Serum levels of histamine, total IgE and kiwifruit-specific IgE have also been determined.
Serum ACE levels were significantly higher in patients with systemic allergy to kiwifruit than those with isolated oral allergy ($p = 0.001$). Levels were significantly higher in patients with severe systemic allergy, and significantly lower in those with isolated oral allergy, than in control subjects ($p=0.05$ and 0.04, respectively). Furthermore, serum ACE levels showed a significant positive correlation with mast cell carboxypeptidase ($p=0.002$). Levels of kiwifruit-specific IgE were significantly higher in patients with systemic than isolated oral allergy to kiwifruit ($p=0.04$).
High baseline serum levels of ACE and perhaps mast cell carboxypeptidase may be associated with severe systemic reactions to kiwifruit. Low levels of serum ACE may be associated with oral allergy syndrome.

O32

Mast cells and the gastrointestinal tract

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Withdrawn

O33

Positive and negative role of mast cells in an experimental model of autoimmune glomerulobnephritis

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Mast cells are a heterogeneous population of cells that are exclusively localized to tissues. While they have been known especially for their role in IgE-mediated allergic disease many studies have also shown their implication in other inflammatory and autoimmune diseases. Besides these negative functions, positive roles of mast cells are also increasingly recognized. In my presentation, I will discuss our results on the role of mast cells in an experimental model of autoimmune glomerulonephritis induced by the injection of antibodies directed to the glomerular basement membrane. Using mast cell-deficient mice, we and others have shown that mast cells play a beneficial role in an accelerated model of disease. This may involve their capacity to initiate tissue repair or to impact on the autoimmune response. However, in more slowly developing disease, mast cells rather show a detrimental role. Analysis of the specific mast cell mediator mMCP-4, the counterpart of murine chymase evidenced a detrimental role of this protease, which may promote the inflammatory response and fibrosis. These data indicate that the physiological action of mast cells involves a complex relationship that highly depends on the physiological context including timing, interaction of mast cells with their environment and the type of mediators mobilized.

O34

Mast Cells Interactions with their Microenvironment in Allergic Inflammation

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Allergic inflammation (AI) is a complex phenomenon consisting usually of two main phases, the early and the late one. Mast cells are the main recognized effector cells of the early phase. Moreover, we have shown that mostly by their cross-talk with eosinophils ("the allergic interface") they also actively participate in the late phase and when the response becomes chronic. Mast cells also take active part in the remodeling of this phase by interacting with the fibroblasts. It is well accepted that bacterial infections can modulate the outcome of AI. We have postulated that bacteria might do this by their specific interactions with the mast cells. In the present study, we therefore have investigated *in vitro* the interactions between human cord blood derived mast cells (CBMC) and the gram+ bacteria *S. aureus* which often colonizes tissues undergoing AI. We have found that *S. aureus* displays a high adherence as well as invasive and survival abilities in the mast cells. TLR2 and CD48 are involved in these processes that cause IL-8 and TNF-alpha release by the infected mast cells. Moreover, the internalized bacteria not only survived but also multiplied. The uptake of *S. aureus* by mast cells may therefore account for the virulence of the bacteria in diseases associated with atopy in which there is also an increased number of mast cells. Overall, this study provides, for the first time, evidence of a direct interaction between *S. aureus* and human mast cells and should strengthen the previous observations of the worsening of allergic disease that takes place concomitantly with *S. aureus* infection.

O35

Mast cells and the skin

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Mast cells (MCs) are abundantly located in the skin as in all organs that border the environment. Cutaneous MC populations importantly contribute to various chronic allergic skin conditions including urticaria and atopic dermatitis. In addition, MCs are involved in a large number and variety of non allergic chronic inflammatory skin conditions (e.g. psoriasis). Recently, a number of independent studies have managed to identify and characterize novel functions of skin MCs. These include physiological functions such as the induction of protective innate immune responses to bacteria and parasites, regenerative functions e.g. in wound healing, homoeostatic functions in growth regulation of skin appendages, and disease limiting functions in chronic inflammatory responses including allergic skin inflammation. Interestingly, mast cells appear to be able to adopt different and sometimes opposite (e.g. pro- and anti-inflammatory) phenotypes and functions at different steps and times during the same process or disease. Here, we present a selection of these recent discoveries and discuss underlying mechanisms including the role of histamine.

O36

Regulation of mast cell survival in health and disease

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Two characteristic features of mast cells are their longevity and their capacity to regranulate after degranulation. Mast cell survival is facilitated through activation of cell surface receptors. Stem cell factor (SCF) binds to Kit and initiates a survival program that involves suppression of the pro-apoptotic protein Bim, both on a transcriptional level and by phosphorylation of Bim that leads to degradation of the protein. Activation-induced mast cell survival (AIS) is facilitated by Fc-receptor activation that causes an upregulation of pro-survival proteins of the Bcl-2 family, such as Bcl-XL, Mcl-1 and A1/Bfl-1. We have found that A1 (mouse)/Bfl-1 (human) is of particular importance for AIS. Mast cells deficient in the A1 gene or human mast cells where Bfl-1 expression has been suppressed by siRNA do not exhibit AIS. This knowledge about the mechanisms regulating mast cell survival could hypothetically be used to induce mast cell apoptosis in mast cell disorders including mastocytosis or allergy. By increasing the levels of Bim in mast cells we have been able to induce apoptosis in these cells, even in the presence of SCF or in neoplastic mast cells with D816V c-kit mutations. Small molecular inhibitors acting on the prosurvival Blc-2-family members induce mast cell apoptosis and also prohibit activation-induced mast cell survival. In summary, we have identified targets that can be used to induce mast cell apoptosis *in vitro* and *in vivo*. Future research will provide evidence if this knowledge can be transferred into new therapies to treat mast cell associated diseases.

O37

On the interaction of H_1-antihistamines with blood platelets and neutrophil leukocytes.

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Background: H_1-antihistamines (H_1-AH) represent a heterogenous pharmacological entity belonging to the group of organic cationic amphiphilic drugs (CAD). Interactions between CAD and blood cells could result in side and adverse drug reactions, which in case of side effects need not be negative.
Results: Depending on their physico-chemical properties, H_1-AH (e.g. phenyramines) dose-dependently inhibited blood platelet aggregation stimulated both at the receptor site (thrombin) as well as by stimuli bypassing membrane receptors (A23187) *in vitro*. The rank order of potency in which the drugs tested inhibited thromboxane formation correlated well with the decline in molar refractivity of the drugs. The increase in partition coefficients as well as 5-100 times higher inhibitory activity of halogenated derivatives suggests that phenyramine halogenation increased the availability of the drug for lipophilic as well as electrostatic interactions with platelet membranes, and that membrane interactions play an important role in the inhibitory effect in platelet functions The inhibition of arachidonic acid liberation from platelet membrane phospholipids and malondialdehyde formation indicated that effective H_1-AH might interact with platelet cytosolic phospholipase A_2.
Human polymorphonuclear leukocytes (PMNL-neutrophils) have a large homogeneous population of H_1 receptors of moderate affinity in a large number. By means of amplified chemiluminescence (CL) technique, we demonstrated that activated PMNL responded with a respiratory burst accompanied by both extra- and intracellular generation of reactive oxygen metabolites. Histamine (HA) and the H_1-AH tested significantly decreased both the extra- and intracellular component of stimulated CL. Interaction with enzymes (NADPH-oxidase, myeloperoxidase, phospholipase A_2, etc) or interference with PMNL membrane structure may well result in reduction of the CL signal. Depending on the concentration used, H_1-AH were more effective in inhibiting activated CL of whole blood than HA. In isolated human PMNL, both HA and H_1-AH inhibited stimulated CL dose-dependently, after specific stimulation (PMA and fMLP). It seems likely that the interaction of H_1-AH with PMNL operated both at extra and intracellular level. The fact that Hi as well as H1-AH decreased the respiratory burst indicates that not only Hi receptors but also non-receptor mechanisms could be involved in the reduction of CL.
Conclusions: Extensive therapeutic use and associated side effects have generated a great deal of interest in understanding the nonreceptor interactions with CADs at cellular and molecular level.

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O38

Aims and Perspectives of the COST Action BM0806: Recent advances in histamine receptor H_4R research

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The histamine H_4 receptor (H_4R) is attracting wide and growing interest due to its function in immune responses and its potential therapeutic exploitation in inflammation, pain and cancers. The objective of this Action is to create a formal network of European experts with the goal to foster a multidisciplinary approach to H_4R research, and to focus on the current state of play pertaining to the basic understanding and the huge therapeutic potential of this important new drug target. Sub-objectives include exchange of ideas and data among participating teams to elucidate all issues on the H_4R and its ligands, deployment of reliable methodologies, larger and more ambitious scale collaborations, as well as training and mobility of young scientists. The duration of the Action is 4 years, and originally includes scientists from 10 COST and 2 non-COST countries with competences ranging from chemical synthesis to clinical pharmacology. More than 20 teams constitute 4 interdisciplinary Working Groups (WGs): methodological approaches; H_4R physiological and pathophysiological importance; pharmacological properties of new selective ligands; therapeutic potential of the H_4R and development of new drug candidates. Emerging scientists are housed in all WGs, gender balance is strictly applied and the geographical distribution of the participants and officials reflect the European dimension of the Action. Continuous monitoring and evaluation, based on reports of a core group (CG), WG leaders and the Young Researchers Coordinator (YRC), and on regular and *ad hoc* discussions, is the responsibility of the Management Committee (MC). The dissemination plan includes a dedicated website, meetings, workshops, short-term scientific missions, publications, a best practice template and open fora. Synergy with relevant programs and international organizations/bodies links the Action to industry and standardisation activities, and will attract interest from a wide range of European countries.

P1

Histamine stimulates IL-1beta mRNA expression and IL-1beta secretion from cultured astrocytes

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Histamine contributes to the regulation of major immune functions. Under certain conditions, the activation of the corticotropin-releasing hormone/substance P-histamine axis may actually facilitate inflammation, through induction of IL-1, IL-6, IL-8, IL-18, TNF-alpha and CRP production. In the brain, the astroglial cells represent important targets for histaminergic neurons. Producing several cytokines, astrocytes crucially contribute to development of immune response in the brain. So far, we have demonstrated several interactions in the cultured astrocytes between histamine, pro-inflammatory cytokines and neurotropic factors. In the present study, we determined the influence of histamine on IL-1beta mRNA expression and IL-1beta secretion from the astrocytes.
We used primary cultures of rat neonatal cortical astrocytes, prepared from the brain of Wistar rats. The cells were treated with different concentrations of histamine in the presence or absence of either PKC inhibitor Gö6976 or MAPK inhibitor PD98059, for 24 hours. Real-time PCR and ELISA were used for determination of IL-1beta mRNA expression and IL-1beta secretion, respectively.
The results showed that the treatment of the cells with histamine significantly increases the IL-1beta secretion from cultured astrocytes. The enhancement of IL-1beta secretion is dose-dependent and reaches the maximum at 100 nM histamine. The effect of histamine can be partially blocked by Gö6976 and PD98059 that indicates the involvement of histamine signalling pathway to the nucleus, where histamine can regulate the expression of certain genes. We also found enhanced expression of IL-1beta mRNA in the cells, treated by histamine.
We concluded that histamine is an important regulator of pro-inflammatory cytokines production in astroglial cells, where stimulates IL-1beta secretion and synthesis as well. Consequently, histamine significantly contributes to development of immune response in the brain.

P2

Histamine influence on apoptosis in trophoblast cell cultures.

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It has been demonstrated that histamine plays an important role in the pathogenesis of preeclampsia. Histamine regulates the process of differentiation of trophoblast cells; it also acts as a growth factor in malignant melanoma cells and prevents monocytic apoptosis. Trophoblast research has shown that in pre-eclampsia placentas, trophoblast apoptosis is significantly increased.

The aim of our study was to demonstrate the influence of histamine on the process of apoptosis in human trophoblast cell cultures.

Placentas were obtained after vaginal delivery. Tissue samples were excised from placentas and, with the use of modified Kliman's method, trophoblast cell cultures were established. The cultures were incubated with dexamethasone, as apoptosis inducer, 48 hours prior to apoptosis detection assays. Along with dexamethasone, selected cell cultures were incubated with histamine (1 µmol/l) or histamine (1 µmol/l) and terfenadine - H_1 receptor antagonist - (from 1 to 5 µmol/l). For apoptotic activity detection, and quantitative analysis, we used an ELISA assays. M30-Apoptosense ELISA Kit is based on the M30 monoclonal antibody that binds only the caspase-cleaved cytokeratin 18 formed during apoptosis in trophoblast cells.

Our investigation showed significantly ($p<0.05$) increased apoptotic activity in cultures incubated with dexamethasone, histamine and terfenadine (% of reference value, +/-SEM): up to 113.1% +/-4.33. Cell cultures incubated with dexamethasone and histamine only, showed significantly lower apoptotic activity 90.2% +/-5.17.

We suggest that histamine may inhibit apoptotic activity in trophoblast cell cultures via the H_1 receptor. Thus, histamine may regulate the process of trophoblast differentiation (via integrin aV-b3 expression, as we previously suggested), and influence cell turnover in placenta.

P3

Histamine and the Invasive Phenotype in Irradiated PANC-1 Cells

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E-cadherin-mediated cell-cell adhesion, metalloproteinases (MMPs) expression and activity, and cell migration are crucial features of the invasive phenotype. Ionizing radiation (IR) is known to induce an increase in invasive potential of different cancer cells. We have previously reported that human pancreatic carcinoma PANC-1 cells express H_1, H_2, H_3 and H_4 histamine (HA) receptors and that HA modulates cell invasiveness through H_1R and H_2R.
In this work, we studied the combined action of HA and IR on PANC-1 cells invasive phenotype. Cells were 2 and 5 Gy gamma-irradiated and incubated with HA and specific agonists for different times.
In non-irradiated cells HA increased ROS (reactive oxygen species) production measured by fluorescent dyes as well as cell migration evaluated by wound healing assay. E-cadherin expression was not modified. In cells treated with H_1, H_2, H_3 or H_4 agonists, intracellular MMP-2 protein detected by flow cytometry was significantly diminished while zymographic analysis revealed an increase in MMP-2 activity in conditioned media. Collectively, data suggest an enhanced secretion of MMP2. It is known that activation and secretion of MMP-2 depend on ROS production. Accordingly, in these cells MMP-2 activity was down-modulated by a ROS scavenger (N-acetyl cysteine). In irradiated cells E-cadherin was upregulated, migration was not affected, whereas ROS levels were significantly decreased.
Even though IR did not significantly decrease MMP2 secretion, it was able to counteract the effect of HA and HA agonists when both IR and HA agonists were combined. This combined treatment did not show differences in E-cadherin expression and cell migration compared to each treatment alone.
In summary, IR does not substantially modify the events related to PANC-1 cells invasiveness which were explored in this study, but is able to affect HA response on MMP-2 activity and secretion probably by altering ROS levels.

P4

Use of lipid rafting for the analysis of leukocyte activation by flow cytometry.

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Background: Human leukocyte activation induced by specific and non-specific stimuli is characterized by the formation of lipid rafts defined as lipid-ordered domains that are more tightly packed than the surrounding non-raft phase of the bilayer. These lipid rafts are formed in parallel with profound membrane reorganization
Objectives: Our objective was to analyse the rafting and non-rafting proteins present on the activated and resting basophil membrane and study their use for the flow cytometric analysis of basophil activation.
Methods: Human basophils obtained from samples used for diagnostic cellular tests such as basophil or lymphocyte activation tests were stimulated either by the formyl-methionyl-leucyl- phenylalanine peptide (fMLP), by anti-IgE or by an allergen. After 40 min at 37 C the cells were labelled by different antibodies conjugated to fluorescent dyes such as an anti-IgE FITC, an anti-CCR3 PE, an anti-CD63, an anti-CD203c PE, an anti-11b, annexin V FITC or by cholera toxin FITC. Moreover, several experiments were analysed by the Amnis cytometer, producing a comprehensive picture of the analysed cells.
Results: Anti-IgE or specific allergen elicit a membrane neo expression of CD63 to a high density, which is poorly represented on the resting basophil membrane. Upon IgE-dependant activation some of the markers already present on resting basophil membrane, such as CD203c, are upregulated and others, including IgE/IgE FcεRI receptor and CCR3, are down-regulated and submitted to the formation of clusters as demonstrated by the pictures taken with the Amnis cytometer. For non-IgE-dependant activators such as fMLP, the picture is different, as IgE is not down-regulated whereas CCR3 was down-regulated. As demonstrated using annexin V or the cholera toxin, used for analysing apoptosis, this phenomenon is paralleled by the formation of lipid rafts, gangliosides domains such as GM1 being accessible from the extracellular medium.
Conclusions: Basophil activation leads to membrane events resembling the apoptosis phenomenon. The flow cytometric analysis of these membrane events may lead to protocols used for allergen-induced activation and, may allow a significant increase of cellular tests sensitivity particularly for drugs allergy diagnosis for which the usual protocols using CD63 alone are insufficiently sensitive.

P5

Is Histamine Dependent Downregulation Of Fibulin Universal Or Cell Line Specific?

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Fibulin-5 (FBLN5) is a small secreted glycoprotein of fibulin family of ECM proteins involved in cell-cell and cell-matrix communications. It is a multifunctional signaling molecule that regulates cell proliferation and cell motility. It plays an important role in organogenesis, fibrogenesis and in tumorigenesis (e.g. kidney, breast, colon and ovary: briefly tumors with metastatic potentials). It can supress or promote tumorigenesis in a context-dependent manner. FBLN5 expression is down-regulated by VEGF, oncogenic Myc and IL-1β while it is up regulated by its master enhancer TGFβ1. FBLN5 expression has been down regulated by histamine acting on H1 receptor in experimental mouse melanoma model established by our research group. Recently FBLN5 mRNA and protein expression were studied using real time PCR and Western blot method in H_1 and H_2 receptor antagonists (loratidine and famotidine) treated human melanoma and colon cancer cell lines as well as in mouse dermatofibrosarcoma (DFS) cell culture established by our group. Low FBLN5 expression was found in primer melanoma cell line WM35 and a higher FBLN5 expression was detected in metastatic cell lines HT168 and M1/15 without correlation to H_1 or H_2 receptor antagonist treatment, while no differences were found in human colon cell lines WiDr, HCT116 and HT29. H_1 receptor antagonist loratidine caused an elevated FBLN5 expression in DFS cell culture from HDC knock-out mouse, (DFSKO) while this effect was missing in DFSWT, the cell culture from wilde type mouse. Western blot results further support these findings. Adhesion ability of primer melanoma cells was higher than this ability of metastatic ones using an impedance based measurement method. According to our results, histamine has cell type dependent action on FBLN5 expression. Differences in its action on melanoma, colon and DFS cell cultures partially could be explained by the different histamine receptor pattern and by the other regulatory molecules.

P6

Histamine increases neuronal clonogenicity and induces expression of deep cortical layer markers after differentiation of neural stem cells.

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During central nervous system development, histamine (HA) concentrations are high and this correlates in time with high rates of neurogenesis. Between embryonic days (E) 13-18 in rat, HA and its synthesis enzyme L-histidine decarboxylase can be detected in rhombencephalon, choroid plexus and adjacent to the third and fourth ventricles (Auvinen and Panula 1988; Vanhala et al. 1994). We recently reported that HA, by H_1 receptor (H_1R) activation, increases 3-fold neuronal differentiation of cortical neural stem cells (NSC; Molina-Hernandez and Velasco, 2008). Here we analyzed the effect of 100 microM HA on cortical NSC clonal cultures expanded during 7-8 days with basic FGF (bFGF), followed by 6 days of differentiation without bFGF. We performed immunocytochemisty experiments to detect neuronal (MAP2+) and glial (GFAP+) cells. This clonal analysis revealed that addition of HA significantly increases the number of clones by 191 ± 16 % relative to control, and also increases the proportion of colonies containing neurones from 26.2 ± 6.2 % (control) to 73.3 ± 3.3 % (100 microM HA). Both effects were reversed by co-incubation of HA with 1 microM chlorpheniramine (H_1R antagonist). In other sets of experiments, molecular analysis of different neuronal phenotypes was performed in NSC cultures differentiated for 6 days after 4 days of expansion with bFGF. As previously reported, HA induced a 2.6-fold increase in the number of $MAP2^+$ cells relative to control. The presence of FoxP2+ and GABA+ neurones was evaluated in control and HA-treated cells. Our results revealed that 100 microM HA did not affect GABA+ cells, but it promoted a 1.5-fold increase on the percentage of neurons expressing the deep cortical layer marker FoxP2 (from 41.4 ± 3 % to 62 ± 5.8 %). In conclusion, we show that H_1R activation mediates neuronal differentiation on low density NSC cultures, and that HA favours the neuronal deep cortical layer phenotype.

P7

Structural Organization of Mammalian Copper-Containing Amine Oxidase Genes

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Copper-containing amine oxidases (AOC) deaminate primary amines to the corresponding aldehydes, ammonia, and hydrogen peroxide. Besides amine oxidation, AOC proteins have been implicated in leukocyte adhesion and glucose uptake and altered activities have been associated with inflammatory bowel disease, chronic liver diseases, and diabetes. Despite considerable sequence variation, AOC proteins have highly conserved homodimeric structures and contain in their active-site a copper ion and the cofactor 2,4,5-trihydroxyphenylalanine quinone.
Only recently it became clear that mammals possess four genes encoding AOC proteins. AOC1/ABP1 encodes diamine oxidase (DAO), a soluble secretory protein mainly expressed in kidney and intestine that inactivates histamine and other diamines. AOC2 encodes retina-specific amine oxidase (RAO), a plasma membrane protein originally identified in the ganglion cell layer of the retina whose function is presently not known. AOC3 encodes vascular adhesion protein-1 (VAP-1), a peripheral plasma membrane protein with monoamine oxidase activity expressed in vascular endothelial and smooth muscle cells that has been implicated in leukocyte extravasation. AOC4 encodes a soluble VAP-1 homologue expressed primarily in the liver from where it is secreted into the bloodstream to constitute the major part of serum amine oxidase (SAO) activity in most mammalian species.
All four AOC genes have highly conserved structures with 4 or 5 exons. The AOC2, AOC3, and AOC4 genes are tandemly arranged on the same chromosome and not linked with AOC1. In addition to functional AOC1, AOC2, and AOC3 genes, the human genome contains a complete AOC4 gene whose reading frame is interrupted by an internal stop codon whereas the genomes of mouse and rat contain only fragments of an AOC4 gene. Both humans and rodents have comparably low SAO activity that is probably derived from partial proteolytic release of the extracellular fragment of the AOC3 gene product VAP-1.

P8

IL-23 suppresses IL-17E (IL-25) in sensitized human lymphocytes *ex vivo*

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Recently, it was demonstrated that interleukin (IL)-23 is a key factor in the regulation of the Th1 and Th2/Th17 balance in human lymphocytes *ex vivo*. IL-17E (IL-25) was found to be elevated in allergic diseases such as atopic asthma. The aim of this study was to measure cytokines in sensitized lymphocyte cultures and to indicate the specific pathomechanisms in atopy.
Human PBMC from 8 atopic/non-atopic volunteers (4 IgE > 500 IU/ml, 4 normal, age matched controls: < 50 IU/ml, age 21 – 35 two males) were stimulated with anti-CD3/IL-2 (1 ng/ml) in 3-day cultures. Allergic patients were suffering from seasonal hay fever and chronic atopic eczema. IL-23 (10 and 20 ng/ml) and histamine (2.5 µM) were added 4 hours post-plating. Cytokines were measured by enzyme linked immune sorbent assay (ELISA). PBMC differentiation was identified using cytoflow/FACS analyses. $P < 0.05$ was considered statistically significant.
There was evidence that IL-17E as well as IL-4 were constitutively increased in the atopic samples with and without anti-CD3-stimulation. IL-23 suppressed IL-17E production in a concentration-dependent manner with a maximum of 40 % at the end of the lymphocyte 3-day culture. ($p < 0.01$, student t-test) However, IL-17E was elevated in the non-atopic group about 12 % at the end of the culture. This effect was not significant compared to the normal anti-CD3 incubates. Interestingly, IL-23 and histamine suppressed IL-4 secretion after 1 day culture. On the other hand, histamine elevated IFN-g even more effectively in the atopic samples.
It could be concluded that the Th1/Th2 paradigm must be modified to be a Th1/Th2 and Th17 regulatory principle. IL-23 is a key factor in allergy patho-mechanisms.

P9

The lymphocyte transformation test (LTT) in type IV Allergy to chemical substances

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In 2006, the AVE developed a list of about 400 allergens to involve in debate of REACH about risk assessment, management and communication. Up to now human and animal *in vivo* tests are common, but should be replaced by *ex vivo/in vitro* tests. We used the LTT to confirm type IV-allergy (contact dermatitis, drug eruption and other chemical rush) in men. In comparison with the classic microscopic evaluation of LTT, we found the ^{14}C-methionin technique depending on methylation of histones is a very useful and rapid test method without the high risk of microbial contamination. The culture time lasts 3 hours only, instead of 4 or 5 days in other techniques. You also can use frozen lymphocytes 4 or 8 weeks after puncture. We paid close attention to toxic effects of formaldehyde, chloramphenicol, sodium-metham and others. Water-insoluble substances were emulgated and dissolved in DMSO or a special acetone-tenside-mixture to ensure testability.
45 substances were tested. We found 173 positive reactions in 271 samples, first of all in allergic contact dermatitis, erythemato-papulous and fixed drug reaction, but seldom if ever in urticaria and other types of rush. There was no case of nonspecific blastic transformation.

Reference: Göring HD, Schubert H, Schwalm I. Allergie Immunol 1978; 24: 199-202

P10

Histamine Affects AtoSC Two-Component System-Mediated Chemotaxis in Escherichia coli

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Two-component systems (TCS) are involved in essential cellular processes in prokaryotes [1]. Although no histamine biosynthetic enzymes or receptor homologues have been identified in *Escherichia coli*, histamine has been shown to functionally modulate the AtoSC TCS [2] and to affect AtoSC-mediated short-chain poly-(R)-3-hydroxybutyrate accumulation in a Ca^{2+}-dependent way [3] in this symbiotic, potentially pathogenic microorganism. This study sought to investigate the effect of histamine on the AtoSC-mediated *E. coli* chemotaxis. Culture samples of *E. coli* BW25113 (*atoSC*$^{+}$) and BW28878 (Δ*atoSC*) transformed with plasmids carrying AtoSC-related genes [2,3] were grown in swarm agar plates containing 5mM Asp/Ser, 1mM propionate or 2.5% (v/v) glycerol as chemoattractants, in the absence or presence of 0.005-0.5mM histamine. After incubation at 30^{o}C for 8h, histamine alone did not affect *E. coli* motility. However, histamine enhanced the swarming phenotype to Asp/Ser in AtoSC-overproducing cells, yet it had no effect on Δ*atoSC* derivatives expressing either the AtoS histidine kinase or the AtoC response regulator. In contrast, histamine partially reduced the chemotactic response to propionate or glycerol. Interestingly, histamine tended to enhance chemotaxis towards Asp/Ser of Δ*atoSC* cells bearing mutations in either one or both AtoC D55 and H73 phosphorylation sites and circumvented the secondary swarm front pattern elicited by non-mutated Δ*atoSC* cells grown on Asp/Ser. In conclusion, these data provide evidence for a yet undefined role of histamine in AtoSC-mediated *E. coli* chemotaxis, thus leading to new perspectives on the cross-talk between this biogenic amine and bacterial signaling during bacteria-host interactions, adaptation and pathogenicity.

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P11

Ectodomain Shedding and C-terminal Fragment (CTF) Formation of Complement Receptor 2/CD21 in Health and Disease

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As with 2-4% of all cell surface proteins, complement receptor 2/CD21 and the low affinity IgE receptor FcεRII/CD23 are post-transcriptionally modulated by proteolytic cleavage of the extracellular domain (ectodomain), resulting in the release of soluble CD21 (sCD21) and sCD23, respectively. CD21 and CD23 interaction controls IgE production, sCD23-binding to CD21 on basophilic cells triggers histamine release, and sCD21-binding to CD23 activates monocytes. sCD21 and sCD23 circulate jointly in sera of healthy donors. However, their levels are often altered in patients with B cell lymphomas, EBV-infection, autoimmune diseases, and asthma - indicating a dysregulated shedding process in these diseases. Importantly, it has been shown for several surface proteins including Notch, CD44, Alzheimer Amyloid Precursor (APP), and many more, that after shedding the remaining membrane-tethered C-terminal fragment (CTF) is involved in cell signaling and transcriptional regulation.

To gain a better understanding of the mechanisms and consequences of CD21-shedding, we wished to determine whether intracellular signaling competent CD21-CTFs would arise upon ectodomain shedding.

To test this hypothesis, we produced a specific antibody that recognizes the CD21 C-terminal domain. Interestingly, primary human and murine B lymphocytes, various B cell lines, and CD21-transfected HEK293 cells revealed the constitutive presence of CD21-CTFs. Moreover, we define that CD21-shedding does not necessarily result in B cell activation and CD21-CTF accumulation, as e.g. redox stimulation with glutathione leads to increased sCD21 but unaltered CD21-CTF levels.

In conclusion, CD21-shedding modulates immune responses via its surface expression and through sCD21 levels, and probably also by the generation of CD21-CTFs. As the CD21 cytoplasmic domain regulates the proteins' own shedding rates, we suggest the involvement of CD21-CTFs in the fine-tuning of B cell activation, and perhaps direct modulation of IgE responses.

P12

Deficiencies in elements involved in TLR4-receptor signalling in RBL-2H3 cells

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Mast cells (MC) are important effectors in allergic diseases and have recently attracted renewed attention for their role in innate immunity. MC have been shown to respond to components of the bacterial cell wall through ''toll-like receptors'' (TLRs), with the production of pro-inflammatory cytokines (IL-4, IL-13, TNF-α). MC can degranulate through a TLR2-dependent pathway or they can be triggered to release newly synthesised mediators, such as TNF-α, after TLR4 stimulation. The RBL-2H3 mast cell line has been widely used as a model for MC responses to various stimuli including LPS, a TLR4 ligand. RBL-2H3 cells have been reported [1] to produce TNF-α after smooth LPS (sLPS; serotype O55:B5) stimulation, though Frankish and co-workers [2] showed that they were insensitive to the O11:B4 sLPS serotype. In rodents, ligand recognition by TLR4 depends on the particular antigenic properties of LPS and different strains might evoke different responses; in this study the effect of the O55:B5 sLPS on RBL-2H3 cells was re-examined. In addition, the response to a rough variant of LPS (rLPS) was also studied. Rough LPS and sLPS have different ways of interaction with TLR4. While rLPS can bind directly to TLR4, the sLPS requires the presence of the co-receptor, CD14, to trigger TNF-α production. None of the concentrations of rLPS and sLPS used elicited a response. This lack of response raised the question of whether the TLR4 pathway in RBL-2H3 cells is operative or not. TLR4, CD14 and MyD88 protein expression was therefore investigated. While TLR4 surface expression was confirmed, CD14 and MyD88 proteins seemed to be absent. The unresponsiveness of RBL-2H3 cells to LPS in our hands can be attributed to a deficient TLR4 cascade and it is interesting to note a parallel between RBL-2H3 cells and the relatives of mast cells, the basophils, since both share this feature of their biochemistry [3].

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P13

Toward bivalent Acylguanidine-type ligands: highly potent and selective histamine H_2 receptor agonists

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N(G)-Acylated hetarylpropylguanidines represent a new class of potent histamine H_2 receptor (H_2R) agonists [1, 2] with improved pharmacokinetic properties due to substantially (by 4-5 orders of magnitude) reduced basicity. In continuation of this work, we explored the bivalent ligand approach by analogy with the concept described by Portoghese for opioid receptors [3]. The aim of this project was to study the structure-activity relationships and to develop pharmacological tools for the investigation of hypothetical dimeric histamine H_2Rs.
The synthesized bivalent ligands were investigated for H_2R agonism at isolated guinea pig (gp) right atria and in a steady-state GTPase assay using Sf9 cells expressing hH_2R-Gs(alpha)S fusion proteins. H_2R selectivities of selected compounds were determined in GTPase assays using recombinant human H_1, H_3 and H_4 receptors. To study species selectivity and the role of the second extracellular (E2) loop, selected ligands were investigated on different H_2R mutants (hH_2R-C17Y-A271D-Gs(alphaS, hH_2R-C17Y-Gs(alpha)S, hH_2R-gpE2-Gs(alpha)S, gpH_2R-hE2-Gs(alpha)S).
The bivalent acylguanidines turned out to be the most potent H_2R agonists described so far (up to 4000-fold more potent than histamine) and to be highly selective for H_2R. Due to insufficient spacer length of the most potent ligands for simultaneous interaction with both binding pockets of a hypothetical H_2R dimer the gain in potency is presumably due to the interaction with an additional binding site at the same receptor molecule. Investigations on mutant H_2Rs confirmed the key role of the non-conserved Tyr-17 and Asp-271 in TM1 and TM7 in the gpH_2R for species-selective H_2R activation and suggest that the E2 loop does not participate in direct ligand-receptor interactions.

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P14

Involvement of histamine and histamine H_2 receptors in nicotinamide-induced differentiation of human amniotic epithelial cells (HAEC) into insulin producing cells.

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Histamine plays a role in various cellular functions, including cell differentiation. Human amniotic epithelial cells (HAEC) maintain stem cell-like characteristics with ability to differentiate *in vitro* into all three germ layers: endoderm, mesoderm and ectoderm. Histamine H_1 and H_2 receptors are expressed on HAEC. In this study, we examined the influence of histamine, and selective H_1 and H_2 antagonists on the generation of pancreatic islet beta-like cells from HAEC in culture.
HAEC were isolated from the amnion after term pregnancies (N=12) and cultured for 14 days in normoxia in 24-well culture plate inserts (1.0 million cells per well) in Ham's F12 and Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and nicotinamide (10 mM). Altogether, 72 cultures were established. Histamine (100μM) effects were investigated with the histamine H_1 and H_2 receptor antagonists, mepyramine (10μM) and cimetidine (10μM), respectively. After 7 and 14 days, the mean concentration of C-peptide (MCCP) in the culture media was measured (human c-peptide ELISA kit, Chemicon Int.) as a marker of the secretion of insulin and pancreatic differentiation of HAEC.
MCCP in the culture media were approximately 3-fold higher on day 14, compared to on day 7. Histamine produced significant ($p<0.05$) increase in MCCP, and more evident differences in this increase were observed after 7 days of culture than after 14 days. The mean percent increase ±SEM of MCCP amounted to 142.19±21.7 and 79.03±12.35, compared to the controls on day 7 and 14, respectively). Histamine H_2 receptor blockade significantly reduced histamine-induced increases in MCCP, both on day 7 and 14 by 88.7±14.3 and 39.2±12.4 (%±SEM, respectively). Histamine H_1 receptor antagonist did not significantly affect MCPP in all cultures investigated.
In conclusion, nicotinamide-induced pancreatic differentiation of HAEC into beta-like cells may be augmented, probably at a very early stage, by histamine acting via H_2 receptors.

P15

Tritium-labeled N1-[3-(1H-Imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294), a high affinity histamine H3 and H4 receptor radioligand

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The histamine H_3 receptor is mainly found in the central nervous system where it acts as a presynaptic receptor modulating the release of histamine and other neurotransmitters. Antagonists of the H_3 receptor are investigated in clinical trials as potential drugs for the therapy of narcolepsy, Alzheimer's disease, epilepsy or ADHD [1]. The fourth histamine receptor subtype is expressed on cells including eosinophils or mast cells and is discussed as a possible target for the treatment of asthma, rheumatoid arthritis and pruritus [2].
To label these histamine receptor subtypes radioligands are powerful pharmacological probes. This study reports the synthesis and pharmacological characterization of tritium-labeled N1-[3-(1H-imidazol-4-yl)propyl]-N2- propionyl-guanidine ([^{3}H]UR-PI294, specific activity 41.8 Ci/mmol), a novel and readily accessible radioligand for the human histamine H_3 receptor (hH_3R) and H_4 receptor (hH_4R). [^{3}H]UR-PI294 displays high affinity for both histamine receptor subtypes (K_D (hH_3R = 1.1 nM, K_D (hH_4R = 5.1 nM). Binding constants determined for H_3R and H_4R reference ligands were consistent with data reported in the literature, confirming [^{3}H]UR-PI294 to be a suitable radioligand for the determination of the affinities of unlabeled H_3R and H_4R ligands. As there is a lack of selectivity between hH_3R and hH_4R, the radioligand is primarily valuable for application in recombinant systems expressing only one histamine receptor subtype. However, H_3Rs are not expressed in cells such as mast cells or eosinophils, where H_4R is mainly located. Therefore, because of the more than 100-fold selectivity over the hH_1R and hH_2R subtypes, [^{3}H]UR-PI294 may also be applicable for labeling of hH_4R in these native cells devoid of hH_3Rs [3].

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P16

[^{3}H]A-349821 Is a Useful H_3 Receptor Antagonist Radioligand In Revealing *In Vivo* Receptor Occupancy of Cognitive Enhancing H_3 Receptor Antagonists.

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[^{3}H]A-349821 is a histamine H_3 receptor antagonist radioligand that was assessed as a radiotracer for determining the *in vivo* receptor occupancy of H_3 receptor antagonists and relating blood exposure levels for H_3 receptor occupancy to efficacy in preclinical models of cognition. Differences in [^{3}H] A-349821 levels in isolated rat cortex versus the cerebellum, a brain region with low levels of H_3 receptor, were used to determine i*n vivo* cerebral cortical H_3 receptor occupancy. Comparisons were made to relate H_3 receptor occupancy to blood levels and efficacy in the 5-trial inhibitory avoidance (IA) response in Spontaneously Hypertensive rat pups, a preclinical model of cognition. [^{3}H]A-349821 (1.5 μg/kg) penetrated into the brain and cleared more rapidly from cerebellum than cortex where [^{3}H] A-349821 levels were optimally 2-fold higher in the latter. Cortical H_3 receptor occupancy by [^{3}H] A-349821 was saturable with a binding capacity consistent with rat cortex membrane *in vitro* binding. ABT-239 inhibited 50% of H_3 receptor occupancy at 14 ng/ml blood, levels that also improve cognitive performance in the 5-trial IA model. Other H_3 antagonists also showed good correlation between blood levels for receptor occupancy and cognitive effects. [^{3}H]A-349821 provided valid measures of *in vivo* H_3 receptor occupancy that may be helpful in guiding and interpreting clinical studies of H_3 receptor antagonists.

P17

Antagonistic activity and selectivity of fluorescent histamine H2 and H3 receptor ligands

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Fluorescent probes are attractive complementary and alternative tools to radioligands for the study of drug-receptor interactions. As part of a program to develop new fluorescence-based methods, such as flow cytometric techniques or assays in the microtiter plate format, we developed prototypical fluorescent ligands for histamine H_1 and H_2 receptors (H_2R) [1, 2]. Stimulated by these results, we extended our project to the H_3 receptor (H_3R). Fluorophores showing emission over 630 nm are superior to overcome problems associated with formerly used dyes, in particular an overlap with autofluorescence of cells used in functional assays. Moreover, the application of such dyes leads to an improved signal-to-noise ratio. Aiming at new fluorescent high-affinity ligands, we used aminopotentidine-type H_2R antagonists as a starting point. Additionally, squaramide-type H_2R antagonists and 3-[4-(piperidin-1-ylmethyl) phenoxy]propanamine-like H_3R antagonists were synthesized and linked with cyanine-, bodipy- and pyrylium dyes via amino-functionalized alkyl spacers of different chain lengths. Pharmacological activities and selectivities were determined in steady state GTPase assays according to standard protocols using membranes of Sf9 insect cells expressing human HRs [3]. The most potent compounds showed antagonistic activities in the nanomolar range at the H_2R and/or the H_3R. The fluorescent probes were successfully applied to visualize receptor binding and to perform competition studies on HEK 293-Flag-hH_2R-His6 cells and HEK-293-hH_2R-Gqs5-HA cells using confocal microscopy and flow cytometry. The results suggest that the synthesized fluorescent H_2R and H_3R antagonists are valuable tools to quantify the affinity and activity of HR ligands in cellular assays.

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P18

Search for Novel, Highly Affine Histamine H_3 Receptor Ligands with Fluorescent Properties

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Histamine H_3 receptors are constitutively active Gi-protein coupled receptors described as presynaptically located auto- and heteroreceptors, that modulate the levels of histamine as well as that of other neurotransmitters such as: ACh, NA, 5-HT. Therefore, blockade of these receptors could be useful in the treatment of different CNS disorders [1, 2] Fluorescent ligands have been in use since the mid-70s, mainly as histological stains [3]. Recent years has brought new applications for these ligands. One of the most topical areas in this field is G-protein coupled receptors [3].
Within the present work, (cyclic)thiourea propoxy derivatives were obtained evaluating the influence of the thiourea group on histamine H_3 receptor affinity. The novel compounds were tested for histamine H_3 receptor affinity *in vitro* in a binding assay for the hH_3 receptor stably expressed in HEK-293 cells. Additionally, the compounds were also tested for histamine H_4 receptor *in vitro* affinity on SF9 cells stably expressing hH_4 receptor co-expressed with different G-protein subunits. As a part of our research, we focus on non-imidazole histamine H_3 receptor fluorescent ligands. The novel fluorescent histamine H_3 receptor ligand was obtained using 4-nitrobenzo[c][1,2,5]oxadiazole (NBD) as a fluorescent moiety. Pharmacological *in vitro* evaluation proved its high affinity at histamine H_3 receptors (Ki = 0.11 nM) as well as promising fluorescent properties. Therefore, this ligand might provide a novel, useful pharmacological tool. The (cyclic)thiourea propoxy derivatives showed lack of affinity at H_3- and weak affinity at H_4 receptors. It could be concluded, that introduction of the (cyclic)thiourea group is not tolerated for both histamine H_3 and H_4 receptors in this series.

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P19

2-CYANO-1-[4-(1H-IMIDAZOL-4-YL)BUTYL]-3-[(2-PHENYLTHIO)ETHYL]-GUANIDINE (UR-PI376): a potent and selective histamine H4 receptor agonist

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Up to now, there is little information about the function of the human histamine H_4 receptor (hH_4R), although, the results of different investigations suggest the hH_4R to play a crucial role in immunological and inflammatory processes [1]. Therefore, selective ligands – including agonists – are needed to pharmacologically characterize and to further explore the (patho)physiological role of the hH_4R.
More detailed pharmacological investigations of N(G)-acylated imidazolyl-propylguanidines – originally developed as H_2R agonists with reduced basicity [2, 3] revealed that several of these compounds are also (partial) hH_3R and hH_4R agonists with potencies in the nanomolar range. This unexpected result prompted us to synthesize related compounds with the aim to gain access to selective hH_4R agonists and to develop such compounds as pharmacological tools.
Exchange of the acylguanidine moiety with a non-basic cyanoguanidine group provided a series of potent hH_4R agonists devoid of agonistic activities at other HR subtypes. Potencies and intrinsic activities of these compounds were determined in steady-state GTPase activity assays using Sf9 insect cells expressing the respective hHR subtype.
UR-PI376 (2-Cyano-1-[4-(1H-imidazol-4-yl)butyl]-3-[(2-phenylthio)ethyl]guanidine) turned out to be the most potent and selective hH_4R agonist in this series (EC_{50} = 34 nM, E_{max} = 0.93) displaying a more than 25-fold selectivity over the hH_3R and negligible activities at hH_1R and hH_2R.

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P20

Functional characterization of (1H-IMIDAZOL-4-YL)ALKYL derivatives at histamine H4 receptor

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Histamine as a ubiquitous chemical messenger mediates its physiological functions mainly by acting at four different GPCR subtypes. Histamine-induced inflammatory conditions, i.e. allergy, asthma and autoimmune diseases can only partly be treated with histamine H_1 receptor antagonists. The identification of the histamine H_4 receptor and its distinct expression pattern on various immune and inflammatory cells underline its potential role as a novel attractive target in medicinal chemistry. Selective H_4 receptor ligands and/or synergism of histamine H_1 and H_4 receptor modulation may be more effective in the treatment of inflammation, allergy, asthma and immunological disorders than previous ones.[1]

Histamine H_3 and H_4 receptors show greatest homology of histamine receptor subtypes. Imidazole-based ligands often show great overlap in binding properties. Nevertheless, remarkably different structure-activity relationships for histamine analogues and imidazole derivatives can be observed in some compound series.

Based on our in-house library screening we expanded ligand binding data and functional properties at human H_4 receptor of imidazole-based ligands structurally defined as amides and carbamates, which have previously been described as potent H_3 receptor antagonists [2,3]. Characterization at the H_4 receptor has been performed by *in vitro* radioligand binding studies and [^{35}S]GTPγS binding experiments using membranes from SF9 cells expressing the human H_4 receptor. Most carbamate and amide derivatives with (1H-imidazol-4-yl)alkyl moiety maintained affinities at H_3/H_4 receptors, whereas some slight structural variations in this core structure cause decreased affinities. Most interestingly, these imidazole-based ligands showed a broad range of efficacy from full agonist to inverse agonist/antagonist properties raising the chance for increased functional selectivity.

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P21

Expression and function of histamine H_4 receptor on human memory Th-17 cells.

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Background: The most recently identified histamine H_4 receptor (H_4R) is functionally expressed on human CD4+ Th2 polarized cells. IL-17-producing T cells (Th17 cells) represent a separate CD4+ T cell subset, distinct from Th1 and Th2 cells. The effector cytokine IL-17 has been implicated in host defence against pathogens and promotes autoimmune pathology. Less data are currently available about expression, regulation and function of H_4R on these cells.
Objectives: The expression and regulation of H_4R on Th17 cells were assessed. Furthermore effects on cytokine release after stimulation with histamine and H_4R agonists were investigated.
Methods: To polarize human CD45RO+ cells into Th17 cells, three cytokine combinations were used. Purified CD45RO+ cells were activated by CD3/CD28 ligation and stimulated with i) TGF-ß+IL-6, ii) IL-1ß+IL-6, iii) IL-1ß+IL-23.
H_4R expression was measured by real time LightCycler RT-PCR and FACS analysis. Cytokine release was determined by quantitative LightCycler RT-PCR and ELISA.
Results: The polarization of human memory Th17 cells was induced by stimulation with all three cytokine combinations. Whereas the Th17 cells which developed in response to TGF-ß and IL-6 yielded the highest amount of the specific Th17 effector cytokines IL-17, IL-22 and TNF-a, H_4R mRNA and protein could be detected in activated CD45RO+ cells and the three subsets of Th17 cells. Furthermore, a significant up-regulation of H_4R protein on the TGF-ß+IL-6 stimulated Th17 cells as compared with the activated CD45RO+ cells was observed. Stimulation of this Th17 cell subset with histamine resulted in a significant up-regulation of IL-22 mRNA. Selective stimulation of the H_4R with 4MH led to an up-regulation of IL-17 mRNA on the Th17 cells differentiated by IL-1ß+IL23.
Conclusions: H_4R is functionally expressed on Th17 cells. This may have implications in diseases where histamine and Th17 cells play a role such as autoimmune pathology.

P22

Detection and Function of H_4R in Spleen- derived Dendritic Cells

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Dendritic cells (DC) are the major antigen-presenting cells that are specialized in the capture, transport, processing and presentation of antigens. During these processes numerous mediators may influence the functions of DC. One of these molecules is histamine, released from neighbouring mast cells, basophil granulocytes and from the DC themselves. Histamine H_4 receptor (H_4R) mRNA expression has been recently described in murine DC by our research group and also by others. However, H_4R protein expression, distribution and its function has not been studied in DC thus far.
We examined the presence of H_4R protein in DC using different molecular methods. After the detection of protein, we investigated the putative H_4R mediated functions. In all experiments DC were separated from mouse spleen using the Miltenyi CD11c microbead kit. H_4R protein expression was investigated by two different methods: immunoblotting technique and flow cytometry. We tested the regulatory effect of histamine via H_4R on specific physiological processes, including migration and antigen presentation of DC. *In vitro* migration was studied in a Transwell system. In order to investigate DC antigen presentation, we used a 5/8 E8 T cell hybridoma cell line, specific for human aggrecan peptide, applied as antigen. IL-2, produced by these hybridoma cells was measured by ELISA.
We could prove the existence of H_4R protein both by flow cytometry and Western blot technique. Furthermore, we concluded from our migration studies that neither different concentrations of histamine nor a H_4R agonist (4-methyl-histamine) influenced the migration capacity of DC. However, we found that another relevant DC function, antigen presentation was significantly increased after treatment with the H_4R antagonist (JNJ7777120).
Taken together, our data indicate that antigen presentation was regulated through the H_4R, while migration of DC was unaffected.

P23

Search for Histamine H_4 Receptor Ligands in the Group of 4-Methylpiperazine Derivatives.

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Histamine possesses an important role in a large number of physiological and pathological processes by acting through a group of four G-protein coupled receptor subtypes: H_1, H_2, H_3 and H_4. The human histamine H_4 receptor (hH_4R) has been recently discovered independently by several research groups [1,2]. The hH_4R shares some homology to the histamine H_3 receptor and is preferentially expressed on the hematopoetic and immune cells (basophils, eosinophils, mast cells and dendritic T cells), suggesting its role in inflammatory and immunological processes [2,3]. Therefore, its ligands may provide a novel therapeutic approach for the treatment of immunological diseases [3].

JNJ7777120 (1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine) is a well known, high affinity antagonist at hH_4R, which was shown to possess at least 1000-fold selectivity over H_1, H_2 or H_3 receptors. In our research 4-methylpiperazino amide part of JNJ was considered as a structural basis in the design of novel molecules. Taking into account the structural analogy, it has been assumed that other 4-methylpiperazine amide derivatives may exhibit comparable pharmacological profiles. In the present study, a series of 14 4-methylpiperazino amide derivatives were obtained and tested for their affinities at recombinant hH_4R, expressed in SF9 cells. Evaluated compounds show *in vitro* affinities in the micromolar concentration range. The pKi value obtained for the most potent compound equaled about 6.0.

In conclusion, it has been found that the 4-methylpiperazino amide fragment is not crucial for the high affinity at the hH_4R.

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P24

Histamine H_3 receptor antagonist-induced pruritus can be inhibited by blockade of histamine H_1 and H_4 receptors

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Pruritus is the predominant clinical sign of most allergic skin diseases. Even though histamine has long been recognised as a classical inducer of itch in humans and mice, the specific mechanism of histamine-induced itch is still unclear. The histamine H_1 receptor (H_1R) and H_4 receptor (H_4R) appear to play key roles in the induction of histamine-induced itch, as H_1R and H_4R agonists provoke itch in mice and blocking these receptors inhibits histamine- and also allergen-induced itch. The participation of histamine H_2 receptor in the induction of itch seems to be minor, whereas the involvement of the histamine H_3 receptor (H_3R) is still not clear yet.

In this study, the role of histamine H_1R, H_3R and H_4R in acute itch was investigated in BALB/c mice. Intradermal injection of the highly selective H_3R antagonist tiprolisant (5 – 500 nmol/ site) induced a strong scratching behaviour in mice. Tiprolisant-induced pruritus could be inhibited by systemic treatment with the selective H_4R antagonist JNJ7777120 (15 mg/kg) and the H_1R antagonist cetirizine (15 mg/kg). JNJ7777120 strongly attenuated the scratching response, whereas the efficacy of cetirizine was only moderate. Interestingly, H_3R antagonism did not further enhance allergen-induced scratching behaviour provoked by repeated administration of the contact allergen 2,4-dinitrochlorobenzene.

These results clearly indicate that H_3 receptors are involved in histamine-induced pruritus. Recent studies have shown that H_3R antagonists cause increases in histamine synthesis and release in peripheral tissues. It is suggested that the increased histamine level in response to H_3R antagonism might activate H_1R and H_4R on sensory neurons, which in turn could result in the excitation of histamine-sensitive afferents and, therefore, elicts the sensation of itch.

P25

Differential effects of H_1 and H_4 Receptor Antagonists on the Cartilage Histamine Content in Rats with Adjuvant Arthritis

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Histamine elicits regulatory functions in inflammation and may play a role in chondrocyte behaviour in rheumatoid arthritis [1, 2]. This study aimed to investigate the effects of H_1 receptor antagonist dimetindene on the cartilage histamine content in a rat model of adjuvant arthritis and to compare them with the respective actions of H_4 receptor antagonist, JNJ7777120. Male Wistar rats of 200-250g bw received normal saline (normal), complete Freund's adjuvant (CFA; Sigma Chem Co, MO, USA), 50mg/kg dimetindene maleate (Sigma Chem Co, MO, USA) and/or 10mg/kg JNJ7777120 (J&J, CA, USA) at day 0, as described previously [2]. Following sacrifice at day 20, the histamine levels of the cartilaginous tissue obtained from #9-10 ribs dissected out medial to the costochondral junction were quantified fluorophotometrically [3]. Differences between treatments were identified by non-parametric statistical analyses and Anova. Compared to normal rats, CFA administration resulted in the development of arthritic signs in the animal paws as well as in statistically significant increases in the cartilage histamine content ($p<0.05$). Contrary to the observed increases in JNJ7777120-treated animals ($p<0.05$), dimetindene induced no alteration in the normal cartilage histamine content ($p>0.05$). In the adjuvant-challenged cartilage, dimetindene, but not JNJ7777120, marginally circumvented the CFA-induced increases in histamine levels ($p=0.081$). In conclusion, these results provide additional evidence for the contribution of histamine in the systemic arthritic phenotype. Interestingly, the findings indicate that, in the adjuvant-challenged cartilage, histamine may exert differential effects through the H_1 and H_4 receptors that are currently under investigation.

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P26

UR-60427 a Novel H_4 Receptor Inverse Agonist that Shows Good Efficacy in Rodent Asthma Models

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Histamine H_4 Receptor (H_4R) is mainly expressed on several lymphopoietic cells in humans and rodents and has been proposed to be an interesting target for immunoinflammatory pathologies. Therefore, our objective was the pharmacological characterization of a new H_4R antagonist selected from our Drug Discovery program.
UR-60427 was identified by a binding assay as a potent ligand of the H_4R (Ki= 9 nM), and was further confirmed to be an inverse agonist by a [^{35}S]GTPgammaS assay. It was shown to be very selective not only vs. the other histamine receptors (>300-fold), but also in a wide panel of GPCRs and ion channels. In cellular assays, it was very potent both in isolated eosinophils (IC_{50}= 3 nM) and in whole blood (IC_{50}= 8 nM) assays. *In vivo* studies in asthma models were performed in ovalbumin (OVA)-sensitised and challenged Balb/c mouse by whole body plethysmography. UR-60427 reduced the methacholine increased Penh when administered at 30 mg/kg bid orally during the effector phase to similar levels than 3 mg/kg dexamethasone. UR-60427 was further evaluated in Brown Norway rats that were actively sensitised and challenged with OVA. Animals were treated with 1, 3, 10 and 30 mg/kg oral doses one hour before and four hours after OVA-challenge and several measurements were performed 24h after. All four doses reduced increased pulmonary resistance induced by serotonin in anaesthetised and tracheotomised animals to levels similar to those of dexamethasone. Total and differential (eosinophils, neutrophils, macrophages and lymphocytes) cell counts in bronchoalveolar lavage were almost completely normalised. Histological analysis revealed that eosinophil counts in perivascular and peribronchial areas were dose-dependently reduced, while mediators (TNFalpha, MIP-2 and eotaxin) that were studied in lungs were not modified.
In conclusion, UR-60427 fulfils good conditions to be developed for respiratory allergic pathologies such as asthma and allergic rhinitis.

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Novel Fluorinated Non-Imidazole Histamine hH_3 Receptor Antagonists of the Diamine Class

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Histamine hH_3 receptor exhibits an attractive therapeutical target for the treatment of different CNS related disorders such as schizophrenia, epilepsy, narcolepsy or Alzheimer's disease. Numerous compounds are already in different stages of clinical studies [1].

Fluorine substitution on GPCR ligands has become a widespread and important modification in drug design and development on medicinal chemistry [2]. We investigated fluoro-containing variations on the lead structures of the potent hH_3 receptor antagonists FUB 880 and JNJ-5207852 [3] and some related precursor molecules for potential labelling with fluoro isotopes in late stages of synthesis. Most of these newly designed ligands showed high affinities in nanomolar to subnanomolar concentration range with antagonist/inverse agonist efficacies. Selected compounds were potent *in vivo* with low ED_{50} dosages on per oral application to mice.

Compounds, labelled with radioactive [^{18}F]-fluoride, are used in positron emission tomography (PET) studies for accelerated clinical trials in the CNS. The novel compounds may offer the chance for new pharmacological tools to get information on receptor density changes during treatment, receptor occupations, interactions etc. Effective [^{18}F]-fluorinated PET ligands are used for a faster, more efficient and more complex drug development in preclinical and clinical studies.

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P28

Histamine H_4 Receptor Ligand JNJ7777120 Inhibits Lung Metastases in MDA-MB-231 Xenograft Tumor-bearing Mice

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We have recently reported the presence of histamine H_3 (H_3R) and H_4 (H_4R) receptors in benign and malignant lesions of the human mammary gland with the level of their expression significantly higher in carcinomas. 50% of malignant lesions expressed H_4R, all of them corresponding to metastases or high invasive tumours. In addition, we showed the expression of H_3R and H_4R in breast cell lines and we found that they are the main receptors responsible for the histamine-mediated responses such as proliferation, apoptosis and migration; in MDA-MB-231 cells.
The aims of the present study was to determine the expression of H_4R and to examine the effect of the compound JNJ7777120 on the survival, tumor growth rate, metastatic capacity and molecular pattern of expression of breast cancer *in vivo*. For that purpose, we established orthotopic xenograft tumors of the highly invasive human breast cancer line MDA-MB-231 in immune deficient nude mice.
Results indicate that the H_4R was the major histamine receptor expressed in the xenograft tumors that also exhibited high levels of histidine decarboxilase (HDC), histamine content and proliferation markers. Mice of untreated group displayed a median survival of 60 days, and a tumor doubling time exponential growth of 8 days. Developed tumors were highly undifferentiated and invasive and 90% of animals exhibited several ganglionary and lung metastases. JNJ7777120 treatment, which was daily administered orally (10 mg/Kg), completely inhibited lung metastases while did not modify significantly survival or tumor growth rate. Tumours from treated animals showed a reduced expression of H_4R and HDC.
This preliminary report describes that JNJ7777120 is capable of abolishing lung metastasis offering a novel therapeutic potential of this H_4R ligand for breast cancer treatment.

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Biogenic amines – Histamine intolerance (HIT) diet

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The prevalence of food allergy has received much interest over the past few years, with an estimated 2-4 % of adults and 6 % of children now suffering from some type of food allergy. An adverse reaction to food is often mislabeled as a food allergy. In many instances, it is caused by microbial food poisoning or intolerance to an ingredient in a food. (1) Food intolerance involves the metabolism but not the immune system. Non-allergic reactions to food are often related to biogenic amines (e.g. histamine, tyramine, serotonin) with symptoms (such as headache, rhinitis, respiratory and digestive complaints, eczema) similar to allergies. (2) This study aims to characterize key points of histamine intolerance diets.
Histamine intolerance (HIT) is almost not genetically induced but acquired by an infection of the intestinal mucosa, reduced diamine-oxidase (DAO)-activity induced by medication, consumption of large quantities of food, rich in histamine and other biogenic amines.
Diagnosis: Oral provocation and determination of Histamine, DAO, and Vit B6 blood level when HIT is suspected. In our investigations, we could show that Histamine is produced by microbial spoilage or during intended processing of food of primarily animal origin in spoilt fish, (esp. tuna and mackerel), salami and raw ham, long ripened cheese, alcoholic beverages such as red wine and beer. Furthermore, it was shown that strawberries, citrus fruits, tomatoes, seafood, and alcohol are histamine liberators. Furthermore, several drugs (e.g. ACC, ambroxol, theophyllin) are able to inhibit DAO. This may induce an increased secretion of histamine. Some antirheumatics and analgetics are also histamine liberators.
It can be concluded that excessive supply of histamine has to be reduced by diet and can be reduced additionally by drugs, nutrients and supplements, e.g. nutrition: (hist)amine-poor diet; medication: (histamine-receptor blockers); food supplements in individual cases: (Vitamin B6 and C, DAO, buffering salts). In proven deficiency of vitamin B6 and a diet rich in protein a substitution of pyridoxine is useful; food industry should be requested to lower the histamine content by selective control of manufacture and use of histamine-poor technologies, as far as possible, poor histamine content as measure of quality.

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P30

Histamine-mediated Biological Responses in WM35 Human Melanoma Cells

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Malignant melanoma is a rapidly spreading skin tumour with a very high invasive capacity and the incidence continues increasing globally. Melanoma cells, but not normal melanocytes, contain large amounts of histamine that has been found to accelerate malignant growth. The expression of the histamine H_1, H_2, and H_3 receptors in melanoma cell lines has been previously reported. In addition, histamine modulates proliferation in a dose-dependent manner in WM35 cells. The aim of this work was to investigate the presence of histamine H_4 receptor (H_4R) in WM-35 cells (human primary melanoma cell line), and its associated biological processes. The expression of H_4R was analyzed by RT-PCR and immunocytochemistry. To characterize the biological responses, we evaluated cell proliferation by the clonogenic, crystal violet and MTT cell viability assays. In addition, cell senescence and differentiation were determined by beta-galactosidase enzyme assay and dopa oxidase activity, respectively. Apoptosis was studied by Annexin-V staining and flow cytometry. Results indicate that WM-35 cells express H_4R at the mRNA and protein level. By using specific histamine agonists and antagonists, we determined that the inhibitory effect of histamine on proliferation is in part mediated through the stimulation of the H_4R (VUF 8430 IC_{50} =1.9 μM, Clobenpropit IC_{50} = 1.7 μM). The decrease in proliferation was associated with an induction of cell senescence and an increase in melanogenesis, which is a differentiation marker of these cells. To our knowledge, this is the first report that describes the presence of the H_4R in WM35 melanoma cells. We conclude that the H_4R is involved in the regulation of cell proliferation and senescence, which represent key processes involved in tumour progression.

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Ghrelin mediates the stimulatory effect of histamine on ulcer healing in rats

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Background: Ghrelin, an appetite stimulating peptide, which is produced in the stomach, is known to be gastroprotective and to enhance the healing of gastric ulcers. However, the link between ghrelin and histamine, a major gastric acid secretagogue, in ulcer healing is not fully understood. In the present study, we attempted to elucidate the interaction between histamine and ghrelin in the process of ulcer healing. Methods: The chronic gastric ulcers were induced in Wistar rats by serosal application of acetic acid (AA) (ulcer area = 28 mm2). The following groups of rats with AA ulcers were treated with; 1) vehicle (saline) (control); 2) histamine (1 mg/kg-d i.p. or i.g.; 3) the selective blocker of gastrin/CCK2 receptor (RPR 30 mg/kg-d i.p.); and 4) the combination of histamine (1 mg/kg-d i.p. or i.g.) and RPR (30 mg/kg-d i.p.). At day 9 after ulcer induction, the rats were sacrificed and the ulcers were analyzed by planimetry and the gastric mucosal blood flow by H_2 gas clearance method. The mRNA expression of CCK2-B receptors was analyzed by RT-PCR. The expression of ghrelin and iNOS was assessed by Western blot. Results: The treatment with histamine significantly decreased the area of gastric ulcer and this effect was accompanied by an increase in gastric mucosal blood flow at ulcer margin and upregulation of mRNA and protein expression for iNOS, CCK2 and ghrelin in the ulcer area. The combined treatment of rats with AA-induced ulcers with histamine and RPR, the blocker of CCK2 receptors caused a significant attenuation of ulcer healing induced by histamine. Conclusions: 1) Histamine stimulates the ulcer healing and this effect is mediated, at least in part, by increased expression of ghrelin in gastric mucosa; 2) Gastrin acting via CCK2 receptors may mediate the ulcer healing activity of histamine and 3) This amine induced enhancement of gastric microcirculation around the ulcer involves local release of NO.

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Plasma histamine (HA) levels during adjunctive H_1-receptor antagonist treatment with loratadine in patients (pts) with active Inflammatory Bowel Disease (IBD)

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There is growing evidence that mast cells, histamine (HA) and other immuno-mediators may play a role in the pathogenesis of Inflammatory Bowel Disease (IBD). This study evaluated plasma HA levels in 30 patients (pts) with CD and 25 pts with UC during acute phase IBD treatment.
Active IBD pts (CDAI>150, CAI>4) were assigned to receive either prednisolone/5ASA/loratadine or prednisolone/5ASA/placebo over 6 months in a prospective randomized, placebo-controlled pilot trial. A standardized conventional steroid tapering regime (starting with 60 mg/day) over 12-16 weeks was used and the dose of 5ASA (3g/day) kept stable during the study period. Plasma HA levels (mean ± SD) were drawn at clinical visits at month 0, 1, 2, 4 and 6 and after an observation period at month 9. HA was determined by ELISA and is given as ng/ml x m2 BSA (normal values < 0.45).
Cumulative steroid dose over 6 months of IBD treatment was lower in antihistamine-treated CD and UC patients than in placebo treated pts (n.s.) and, interestingly, loratadine-treated pts had some beneficial clinical effects during month 2 - 4. But plasma HA levels did not differ in pts taking loratadine or placebo, neither in CD (MO 0.47 ± 0.7 vs 0.25+0.1, M6 0.28 ± 0.1 vs 0.12 ± 0.1, M9 0.13 ± 0.1 vs 0.15 ± 0.1) nor in UC pts (M0 0.24 ± 0.1 vs 0.37 ± 0.4, M6 0.29 ± 0.2 vs 0.19 ± 0.1, M9 0.26 ± 0.2 vs 0.36 ± 0.4).
This study shows that plasma HA levels in active IBD are mostly within the normal range, show low variation and that low-dose H_1-receptor antagonist treatment as adjunctive therapy does not significantly alter plasma HA levels in IBD. Although loratadine treatment exerted some clinical significant improvements, these benefits on IBD pts are not reflected by plasma HA, suggesting that the H_1-receptor blockade works at the intestinal/local tissue level.

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Recruitment and Activation of Human Basophils in Bullous Pemphigoid: Enhancement of IgE-Mediated Histamine Secretion by Blister Fluid.

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Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease characterized by IgG autoantibodies-directed hemidesmosomal proteins such as BP180 and BP230. However, it is also associated with IgE autoantibodies against these proteins as well as underlying Th2 immunity and histamine release from allergic effector cells. Since basophils readily elaborate Th2-type cytokines and histamine upon IgE-mediated stimulation, the aim of this study was to determine their presence in BP skin and their activation to autoantigens and blister fluids. Basophils were isolated from peripheral blood by Ficoll-density centrifugation and negative selection (magnetic cell sorting). The cells were stimulated with recombinant autoantigens, lysed human keratinocytes, anti-IgE, blister fluids or buffer alone for various periods and mediator secretions were measured either by ELISA (IL-4) or spectrofluorometrically (histamine). Immunohistochemical staining of basophils was performed using the basophil-specific BB1 antibody in skin biopsies, showing their presence in lesional and non-lesional BP skin but not in non-BP donors. Upon stimulation with lysed keratinocyte extracts, basophils from BP patients released 21 ± 6 % histamine and 90 ± 27 pg/million basophils of IL-4. Control donor basophils failed to release these mediators above unstimulated levels but degranulated to keratinocyte extracts following sensitization with BP sera. BP basophils also responded to stimulation with BP230-C1 antigen but only 20% of donors showed any marked response to NC16A. Additionally, diluted blister fluids (1:10) from BP patients stimulated basophil histamine releases by 3-9% above spontaneous levels and considerably enhanced (by 316 ± 112%) IgE-mediated release of the amine. These data demonstrate the recruitment of basophils to BP skin and their activation by blister fluids and certain autoantigens (e.g. BP230), suggesting a substantial contribution of this cell type in the pathogenesis of BP.

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Prospective randomised, placebo-controlled trial of additional loratadine treatment in Inflammatory Bowel Disease (IBD)

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There is growing evidence that mast cells, histamine and other immuno-mediators may play a role in the pathogenesis of IBD. This study evaluated prospectively whether loratadine (10 mg/day) may have a positive effect on the course of IBD under standard acute phase treatment.
30 patients (pts) with Crohn's Disease (CD) and 25 with ulcerative colitis (UC) were assigned to receive either prednisolone/5ASA/loratadine or prednisolone/5ASA/placebo over 6 months. A standardized conventional steroid tapering regime (starting with 60 mg/day) over 12-16 weeks was used and the dose of 5ASA (3g/day) kept stable during the study period. Clinical study parameters were obtained by disease activity scores (CDAI, CAI), serological, immunological parameters and cumulative steroid dose during 6 months treatment (mg/kg body weight).
Cumulative steroid dose over 6 months of IBD treatment was each lower in antihistamine treated CD (56.3 vs 76.4) and UC pts (54.2 vs 56.1) than in placebo treated patients.
Interestingly, after month 2, a clear reduction ($p=0.05$) of abdominal pain was found in both CD (47.4% of pts with pain vs 80%) and UC (22.2% vs 57.1%), while fistula activity was found to be significantly reduced in CD during month 2 and 4 (0% of pts had fistula vs 20%, $p=0.02$). In addition, blood in stools of UC pts was also significantly improved at months 2 and 4 in the loratadine-treated group than in the placebo group (33.3% of pts with blood vs 64.2%, $p=0.03$).
Several other clinical and immunological study parameters including urinary methylhistamine and serum orosomucoid levels did not differ.
This study shows that low-dose H_1-receptor antagonist treatment as adjunctive therapy in IBD exhibits additional beneficial effects in terms of abdominal pain, fistula reduction, loss of blood in stools and thus appears to mediate additional anti-inflammatory effects. The beneficial effect of 10 mg loratadine/day in IBD needs one to two months of treatment and may be further augmented in future trials, when using higher daily antihistamine doses.

P35

Antihistaminic drugs modify casein-induced inflammation

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All known antihistaminics may affect several inflammatory events, including chemotaxis and the survival of eosinophils, release of chemokines and cytokines from different sources, thus highlighting their potential for a modulation of chronic inflammation and immune responses. The aim of the study was to examine effect of H_1-H_4 antihistaminic drugs in acute model of casein-induced inflammation in rat. Level of histamine in blood, chemiluminescence of PMNs and number of granulocytes were measured.
Inflammation was induced by injection of 12% solution of casein into peritoneal cavity of Wistar rats. Rats were treated intraperitonelly with pyrilamine meleate (and histamine H_1 receptor antagonist) (10/mg/kg), cimetidine (and histamine receptor H_2 antagonist) (25mg/kg), thioperamide maleate (a histamine receptor H_3/H_4 antagonist) (2mg/kg) and ciproxifan hydrogenmaleate (a histamine receptor H_3 antagonist) (0.14 mg/kg) twice: 2 hours prior and 4 hours after casein administration.
We found that level of histamine in casein induced inflammation is higher than in control group. Treatment with pyrylamine and ciproxifan additionally increased levels of blood histamine during inflammatory response. Peripheral blood neutrophils from rats with casein-induced inflammation tended to respond less to zymosan stimulation than neutrophils from controls. Selective H_1 and H_3 antagonists injected into rats with casein induced inflammation significantly increased neutrophils response to zymosan ($p<0.01$).
It may be concluded that histamine produced or released into blood in the course of experimental inflammation exerts its effects on the PMN-s via stimulation of H_1 and H_3 receptors.

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The measurement of leukotrienes in urine - diagnostic option in systemic mastocytosis?

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To diagnose a systemic mastocytosis the measurement of the histamine metabolite methylhistamine in urine (UMH) takes place. Beside histamine, leukotrienes seem to play an additional role in the pathogenesis of sytemic mastocytosis. Therefore, the leukotriene B4 and the cysteinyl-leukotrienes C4, D4 and E4 were measured in urine samples of patients with systemic mastocytosis and the values were compared to the UMH values.
The 9 participating patients were divided into a group with high disease activity and one with low disease activity. Leukotriene B4 and the cysteinyl-leukotrienes were measured in 12-hours urine samples by an ELISA, and urinary methylhistamine by tandem mass spectrometry. Subsequently, the values were related to urine creatinine and the body surface (BS).
Patients with a high activity of systemic mastocytosis showed elevated values of the cysteinyl-leukotrienes (mean 51.8 ± 36.2 ng/mmol creatinine x m2 BS). Even patients with low activity had increased cysteinyl-leukotriene values compared to a control group (mean 38.9 ± 39.8 versus15.0 ± 10.3 ng/mmol creatinine x m2 BS). In addition, there was a good correlation to the UMH values and thus to disease activity. The urinary leukotriene B4 values were also increased in patients with systemic mastocytosis in comparison to the control group, but they showed no correlation to the UMH values and to disease activity.
Prospectively, the urinary cysteinyl-leukotriene values could be used as additional parameters in clinical diagnostics and in control of systemic mastocytosis. The transformation of eicosanoids in mast cells seems to be mainly to cysteinyl-leukotrienes. Therefore, using cysteinyl-leukotriene-antagonists and 5-lipoxygenase-inhibitors as supplemental therapy in systemic mastocytosis - beside antihistamines - is to be considered.

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Histamine Intolerance - A Metabolic Disease?

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Despite its diverse physiological functions, histamine is widely known only as a mediator of allergy and inflammation. Along this line the disease concept of histamine intolerance or enteral histaminosis has been introduced to explain various symptoms including gastrointestinal discomfort, urticaria, rhinitis, headache, asthma-like symptoms, and cardiovascular complaints thought to be caused by dietary histamine that is resorbed due to inadequate catabolism in the intestine.
Histamine can be inactivated either intracellularily by methylation of the imidazole ring yielding N4-methylhistamine, a reaction catalyzed by the cytosolic enzyme histamine N-methyltransferase (HMT), or extracellularily by oxidative deamination of the primary amino group yielding imidazole acetaldehyde, catalyzed by the secretory enzyme diamine oxidase (DAO). Enzyme inhibition studies in animals showed that DAO appears to be the principal barrier for the resorption of histamine from the intestinal lumen whereas HMT plays only a minor role. As the expression levels and tissue activities of both enzymes show considerable variation in humans it is conceivable that an increased histamine intake may saturate the intestinal enzymatic barrier function in certain individuals leading to resorption of significant amounts of histamine and histamine mediated adverse reactions.
Since dietary histamine present in foodstuffs is produced by microorganisms in the course of fermentation and spoilage and is usually associated with other biogenic amines exhibiting strong pharmacological activity, adequate diagnosis of histamine intolerance must exclude other causes and should include oral histamine provocation with determination of resorption and associated symptoms. The only therapy for histamine intolerance currently available is avoidance of histamine containing food that will usually also contain low levels of other pharmacologically active biogenic amines.

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Receptive Music Therapy (rMT) and Saliva Histamine Secretion

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Recently, we described responses of receptive music therapy (rMT) on the histamine secretion in blood of atopic and non-atopic young students. Several of the volunteers suffered from certain psychogenic stress before and during blood drawing. Therefore, it was considered to measure histamine secretion from the mouth saliva.
Two matched controls (2 atopics “A” total IgE > 500 IU/ml, typical acute and chronic allergic symptoms like atopic eczema and asthma; 2 non-atopics “NA” total IgE < 50 IU/ml; age 20 – 26 females) were exposed with “good feeling music” for 5 min twice as described previously. [1] Protocol: Once during rMT “adverse food” like fish and vegetables was eaten. It was usually rejected by the test persons. Saliva was collected from the mouth before and after music exposure. Histamine and sIgA were determined using the ELISA-technique (IBL Hamburg). Histamine was additionally measured by routine (OPD-)fluorimetry after HPLC. Significance was defined $p < 0.05$ using student t statistical calculations.
It could be shown that saliva histamine was suppressed only in 2 test persons. Furthermore, sIgA concentrations were in the range 260 – 500 µg/ml and did not alter during the course of the experiments. There were no differences between the A- and NA-matched controls. As described previously, the pulse strokes were significantly reduced in all experiments after “good feeling music” exposure. This observation also applied when the pulse was elevated after eating “adverse food”.
It could be concluded that with respect to the individual differences of stress responses, histamine in saliva is an appropriate tool for measurements of the physiological effects of rMT.

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P39

Ex vivo-Responses of Mixtures of Bacterial Wax esters in Sensitized (S) and Non-sensitized (NS) Human Blood Cell Incubates

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Wax esters are natural protection factors on the human skin. Consequently, they could serve as a useful basis in biotechnological production of cosmetics. The aim of this study was to assess the allergenic potential of mixtures of bacterial wax esters (charges).
The histamine liberation test (HLT) and the lymphocyte stimulation test (LST) were used as immuno-toxicological tests of the wax ester mixtures. A marine Gram-negative bacterium was cultivated under aerobic conditions and 6 wax ester charges were separated as described elsewhere [1]. The HLT was incubated with suspensions of different charges using human PBMC (total IgE: S > 500 IU/ml suffering from typical chronic and seasonal allergic symptoms such as hay fever and atopic eczema and NS < 50 IU/ml without any symptoms; n = 4 age 20 – 26 one male). HLT: PBMC 20 min, 37°C, 1.5 M perchloric acid supernatants, fluorimetry (OPD) after HPLC. LST was performed according to [2].
Only in two wax ester charges (A and D) a significant increase of the histamine liberation (HLT) was measured in NS samples. ($p < 0.05$, student t-test) In the LST, charge C showed 53 % inhibition of the proliferation (MTT-test). This was similar when comparing the sensitized (S) and non-sensitized (NS) incubates. The IFN-γ/Il-4 = Th1/Th2 immuno-toxicological index (normal = 1.0, [2]) was significantly increased (4.1) in the NS charge B. At wax ester concentrations ≤ 2.5 10^{-5} g/l IL-4 was inhibited during the whole incubation time.
Based on this desensitizing effect it could be suggested that the microbial approach applied to produce wax esters can be a benefit of cosmetic manufacturing.

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P40

Saliva Histamine Secretion after *Cynara scolymus* L. (Artichokes) oral challenge

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Nutrition factors can influence saliva secretion. Artichokes (*c. scolymus*) induce saliva production. In this study the histamine concentrations were measured in a group of atopic patients and non-atopic volunteers.
Eight female volunteers (2 allergic patients: IgE > 500 IU, no specific reaction against *cynara scolymus*, atopic asthma and eczema;6 nomergic IgE < 100 IU without any severe symptoms, age 20 – 36) collected 0.5 – 2 ml saliva in acryl tubes (1 h fasting), 2nd day 10 min after mouth cleaning with tap-water, 3rd day after artichoke eating (1/2 cooked flower). Histamine was determined in the cooking water (control) and in saliva after perchloric precipitation. Histamine was measured by OPD-fluorimetry after HPLC and statistical calculations were carried out as described elsewhere [1].
The histamine concentrations in saliva juice were significantly decreased after mouth cleaning with tap-water. (mean normal without cleaning: 5.7 ± 7.2 ng/ml; after cleaning: 2.9 ± 1.3; $p < 0.05$, student t) An activated saliva secretion could be observed after artichoke challenge in all examined volunteers. The taste was defined to be “sweet” (alpha-amylase). In this experiment the histamine concentrations were increased significantly (81 ± 31.6 atopic group, 65 ± 28 ng/ml non-atopic group; $p < 0.01$ student t-test). An average of 0.05 mg per artichoke was measured from the cooking water extracts which could be ignored as background related to the saliva histamine contents. In all experiments the histamine concentrations of the allergic patients were increased compared to the non-atopic group after artichoke challenge. However, there were no significant differences between the allergic patients and the normergic volunteers in the other experiments.
It could be concluded that artichokes did not only induce saliva histamine but also histamine secretion. The saliva histamine concentration was significantly increased in the atopic patients.

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P41

Cryotherapy decreased histamine level in blood of patients with rheumatoid arthritis

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The conventional physicotherapy (electrotherapy, magnetic fields), kinesitherapy and whole-body cryotherapy (plus kinesitherapy) are used to relieve pain and inflammation or to improve function in rheumatic diseases.
The aim of this study was to investigate the effects of different physicotherapies and cryotherapy on biochemical blood parameters of patients with rheumatoid arthritis (RA) and osteoarthritis (OA).
44 patients with RA and 45 patients with OA, according to the American College of Rheumatology (ACR) diagnostic criteria, were enrolled in this study. For the whole period of the study and three month before in all patients, the pharmacological treatment was not changed. Twenty patients with RA and seventeen patients with OA received whole-body cryotherapy at minus 140 – 160 degrees C for 2 to 3 minutes, once daily for four weeks. The second group of patients received conventional physicotherapy for four weeks. Blood levels of histamine, chemiluminescence of PMNs, plasma level of calprotectin, N-acetyl-beta-D-hexosaminidase (NAH-ase) in serum were measured twice: before and 3 month after cryotherapy or physicotherapy.
We showed, for the first time, that cryotherapy reduced significantly ($p<0.001$) histamine levels in blood of patients with RA. The effect is long-lasting (for at least 3 months).The level of histamine in patients with OA was not changed significantly. Cryotherapy also down-regulated respiratory burst of PMNs, calprotectin and NAHase levels but these changes were not statistically significant. In contrast, in groups of patients treated only with physicotherapy and kinesitherapy, we did not observe significant changes in biochemical parameters measured.
It may be concluded that the beneficial clinical effects of cryotherapy in RA patients are in part due to the action on the production, release or degradation of histamine.

P42

Histamine in pericarditis of children with congenital heart malformations

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Congenital heart malformations are the most common heart defects in children, which interfere with a variety of disease processes in which pericardium is often involved. Pericardial involvement is generally manifested as an accumulation of fluid in the pericardial sac or inflammation of pericardial structures.
The aims of the study were to examine the levels of histamine in pericardial fluid and the presence (PCR) and immunolocalization of the proteins participating in releasing and reuptake of histamine in the pericardium of children undergoing heart reconstruction surgery.
We found that the concentration of histamine in the pericardial fluid (from 1.2 to 62.0 ng/ml }was directly dependent on the severity of inflammation. Histamine releasing factor (HRF) was localized only in normal epithelial cells of pericardium, but was not present in degenerating or proliferating epithelium. Numerous mast cells were found in subepithelial connective tissue of pericardium and histamine H_4 receptors were localized only on these cells.
The results suggest that in pericarditis of children with congenital heart malformations, histamine may play an important role, but it does not participate in the proliferation or degeneration of pericardial epithelial cells.

P43

Detection of allergen-specific IgE using the ALLERG-O-LIQ System based on the Reversed-enzyme-allergo-sorbent-test

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Allergen-specific IgEs cause histamine release on mast cells and thus play an important role in the pathogenesis of type I allergies. Beside patient's case history, physical examination and skin prick testing, *in-vitro* tests for the detection of IgE are mandatory for the diagnosis of type I allergies. For the determination of the specific IgE and total IgE concentration in human serum a high number of commercial test systems are available. Most commercial assay systems use immobilized allergens in combination with an anti-IgE enzyme conjugate as reporter system. In contrast, the ALLERG-O-LIQ System follows the reversed-enzyme allergo-sorbent test (REAST) protocol using anti-IgE coated microtitre plates in combination with biotinylated allergens and streptavidin HRP as detection method. This assay architecture results in two purification steps during the assay procedure, namely the purification of the IgE fraction from the patient's samples and the enrichment of IgE binding proteins from the extract. Recent studies have compared the ALLERG-O-LIQ System with Skin Prick Test (SPT) and with the ImmunoCAP® showing a high degree of concordance for most allergens. Several factors such as the presence of specific IgG, the affinity of the specific IgE and the total IgE content may influence the results of certain specific IgE assays. Moreover, the affinity of specific IgE and the density of specific IgE in relation to the total IgE concentration have been discussed as important factors which play a key role in the pathogenesis of type I allergy. This presentation summarizes all important studies on REAST compared to assays based on the classical protocol for the detection of specific IgE.

P44

Regulatory role of histamine in astrocytic NT-3 synthesis

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Astrocytes actively control neuronal activity and synaptic transmission and, by producing various neurotrophic factors, represent an important local source of trophic support in the normal and diseased brain. Our present study showed the ability of astrocytes to synthesis neurotrophin-3 (NT-3), in addition to nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which can be regulated by various monoaminergic neurotransmitters including histamine (HA). The active involvement of multiple histaminergic receptor mechanisms in the regulation of NT-3 production by HA in neonatal rat cortical astrocytes was examined. HA (1 μM) maximally elevated NT-3 levels by 2.1-fold after 6 h of incubation. Its stimulation was partly inhibited either by the H_1-antagonist triprolidine, the H_2-antagonists famotidin and cimetidin, or by the H_3-antagonist ciproxifan. NT-3 levels in astrocytes were increased by the specific and selective H_1, H_2 and H_3 agonists but none of the tested agonists was able to reach the level of HA stimulatory effect. Different activators of basic intracellular histamine receptor second messenger systems (forskolin (20 μM), dibutyryl cAMP (dBcAMP) (100 μM), as well as calcimycin (1μM) (Ca^{2+} ionophore A23187) and phorbol 12-myristate 13-acetate (TPA) (100 nM)) markedly increase the cellular level of NT-3 protein.
Our study confirmed that the synthesis of NT-3 in astrocytes is regulated by histaminergic system and indicate the possible involvement of multiple, complex histamine H_1, H_2 and H_3 receptors and intracellular mechanisms involving the cAMP/protein kinase A pathway, as well as mobilisation of Ca^{2+} ions and activation of protein kinase Ca

P45

Histamine-induced cardiovascular effects in rats after repeated treatment with amitriptyline

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Antidepressant drugs are able to influence cardiovascular regulation acting both centrally and peripherally [1-2]. Our previous studies demonstrate that pre-treatment with amitriptyline (AMI), given 15 min or 1 h before histamine, significantly reduces falls in mean arterial pressure (MAP) and pulse pressure (PP) induced by exogenous histamine in rats [2]. Moreover, AMI shows high capability to reduce an increase in plasma histamine levels after bolus injection of the amine [2] or after injection of the compound 48/80 which releases histamine from the intracellular compartment [3]. In this study, we were interested in pharmacodynamic effects of applied histamine or histamine liberator after consecutive treatment with tricyclic antidepressant We examined cardiovascular reactivity to exogenous histamine and to the compound 48/80 in rats 1 week after termination of pre-treatment with AMI (2.7 mg/kg, ip for 7 days). Measurements [MAP, PP and heart rate (HR)] were followed in ketamine/xylazine-anaesthetised male Wistar rats. In the saline pre-treated group, bolus injection of histamine (10 µg/kg; iv) transiently decreased MAP and PP by 44.3% and 51.42%, respectively, with no significant influence on HR. In contrast, decreases in MAP and PP after the injection of the compound 48/80 (1 mg/kg; iv) lasted to 90-120 min of observation and reached up to 58.5% and 64.12% of the initial, respectively. In AMI-pre-treated groups, MAP and PP changes evoked by histamine and the compound 48/80 were significantly reduced. Present results support findings that tricyclic antidepressants may evoke important additional effects. The question rises whether persons on antidepressive-therapy respond differently to stress conditions that lead to histamine release.

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P46

Histamine uptake into cultured neonatal rat astrocytes is affected by histamine metabolism

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Every neurotransmitter should be inactivated shortly after having been released into synaptic cleft. The subthreshold synaptic concentration of neurotransmitters can be achieved by diffusion into extracellular space, by enzymatic degradation, and by uptake into presynaptic neurons and perisynaptic astrocytes. In our previous work, we have shown that histamine is taken up into astrocytes by two independent processes, electrodiffusion and the active transport. However, the exact nature of this transport, specific or non-specific transporters involved, or role of histamine degrading enzymes in this process remain an unresolved issue.
The aims of our present study were to establish the nature of histamine transport into cultured rat neonatal astrocytes in the presence of foetal calf serum (FCS) and to clarify the role of histamine degrading enzyme, histamine N-methyltransferase (HNMT), in this process.
Histamine uptake into cultured rat neonatal astrocytes showed Km 3.5 +/- 0.8 microM, Vmax of 7.9 +/- 0.3 pmol/mg protein/min and maximal uptake of 488 +/- 57 pmol/mg protein. The addition of 10% FCS into incubation medium significantly decreased the amount of taken up histamine into cultured astrocytes, when higher histamine concentrations were used (304 +/- 15 pmol/mg protein, $p<0.05$, Student independent samples t test). The addition of HNMT inhibitor metoprine (10 nM), significantly increased histamine uptake in the absence or presence of FCS ($p<0.05$), whereas another HNMT inhibitor of, amodiaquine (10 microM), significantly decreased the amount of taken up histamine within astrocytes ($p< 0.05$, Student independent samples t test), only when FCS was added to incubation medium.
These preliminary results indicate that histamine degradation via HNMT affects histamine transport into cultured rat astrocytes, but further studies are needed to clarify this process.

P47

Click chemistry on histamine H_3 receptor ligands and first metal containing antagonists

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Human histamine H_3 receptors (*h*H_3R) are acting as autoreceptors on synthesis and liberation of histamine as well as heteroreceptors, on modulating the release of several other neurotransmitters (e.g. acetylcholine, dopamine, glutamate, noradrenalin, serotonin, GABA). Due to the distinct expression in the central nervous system (CNS) and the involvement in several neuronal functions, e.g. vigilance, attention and learning, the *h*H_3R is an attractive target for the treatment of CNS disorders such as schizophrenia, epilepsy, depression, Alzheimer's disease and sleep disorders[1].
Based on the general construction pattern for a *h*H_3R antagonist, we approached click chemistry as a convenient and efficient way for structural variations. The triazole moiety as a linker to or a replacement for the most common phenylether structure was eligibly achieved by a Huisgen 1,3-dipolar cycloaddition of azides and alkynes under copper-(I)-catalysis[2].
Additionally, synthesis of the first metal-containing *h*H_3R ligands with high binding affinities has been achieved by introduction of ferrocene moieties. The compounds obtained differ in linking elements between the piperidinopropyl and ferrocene moieties. The *h*H_3R binding affinities of the newly designed antagonists range from no effect to subnanomolar concentration ranges. The implemented iron ion on these compounds may be replaced by radioactive $^{99m}Tc(CO)_3$ *via* a double-ligand-transfer reaction[3]. Compounds having these structural features are already used in single photon emission computed tomography (SPECT) investigations.
In our work, we have successfully adopted click chemistry for the convenient synthesis of *h*H_3R ligands. Also, introduction of the iron-containing ferrocene element led to highly affinity *h*H_3R antagonists, with a new metal-containing moiety, which are potential precursor molecules for SPECT investigations.

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P48

The histamine H_3 receptor as a potential antinociceptive target: Effects of selective H_3 antagonists in several preclinical pain models and the involvement of noradrenergic systems

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The histamine H_3 receptor is predominantly expressed in the central nervous system and plays a role in several physiological mechanisms including release of the neurotransmitter norepinephrine. The potential involvement of H_3 receptors in pain processing has recently been suggested in view of the efficacy of H_3 receptor antagonists in preclinical pain models. In the present study, several potent and selective histamine H_3 antagonists of diverse structural type were extensively profiled in multiple pain models. Systemic administration of H_3 antagonist produced full efficacy in reducing pain behaviors. Specific models supported mechanism-based efficacy in rat models involving central sensitization of pain responses. When tested in neuropathic pain models (e. g. spinal nerve ligation and sciatic nerve constriction injury), efficacy was less in the former than the latter. Efficacy of H_3 antagonists in acute inflammatory pain models was also examined. Site-of-action studies were carried out (e.g. by systemic vs. intrathecal administration), where it was found that the antinociceptive effects of an H_3 antagonist were retained after i.t. administration of very low doses. Mechanism-of-action experiments showed that antinociceptive effects could be blocked by the alpha$_2$-adrenergic antagonist. phentolamine. Overall, the results supported that the analgesic efficacy of H_3 antagonism in rat preclinical pain models is likely mediated at spinal levels, and the effects involve activation of the noradrenergic system. Taken together, the data support that selective H_3 antagonism may represent a new and useful mechanistic approach for pain relief.

P49

Severity of seizures and neuronal damage are enhanced in the 9-day-old histamine 1 receptor knockout mice

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The functional disturbance of the central histaminergic neurons is proposed to contribute to the severity of epilepsy. Our recent study indicated that the tuberomamillary histaminergic neurons protect the hippocampus from kainic acid (KA)-induced neuronal damage through H_1 receptors in the organotypic coculture system (Kukko-Lukjanov et al. 2006). We have now further examined the role of H_1 receptor-mediated regulation of KA-induced seizures and neuronal damage in the immature H_1 receptor knock out (KO) and age-matched wild-type (WT) mice.

The severity of the behavioural seizures was significantly enhanced in the 9-day-old (P9) H_1 receptor KO mice when compared to the WT mice at the KA dose of 2 mg/kg. The total duration of the behavioural seizures was significantly longer in the KO mice when compared to the WT mice both at the KA doses of 2 and 3 mg/kg. Moreover, neuronal damage was significantly enhanced in the CA1 region of the hippocampus, thalamus, and retrosplenial granular cortex (RGC) in the KO mouse when compared to the WT mice. Finally, the H_1 receptor antagonist, triprolidine (10 mg/kg) resulted in significantly more severe seizures when compared to the KA-treated WT mice. Triprolidine also significantly increased KA-induced neuronal damage in the septum, thalamus, and RGC when compared to the WT mice.

Our results indicate that H_1 receptor deficiency increases the severity and duration of seizures in the developing mice. In addition, more severe seizures resulted in enhanced neuronal damage in the specific brain regions of the H_1 receptor KO mice. Our results suggest that the H_1 receptor plays an important role in regulating the severity of seizures and seizure-induced neuronal damage in the immature mice.

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J Neurosci. 2006;26:1088-97.

P50

Histamine selectively excites projection neurons in the cerebellar nuclei

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Neuroanatomical evidences have revealed direct histaminergic projections from the tuberomamillary nucleus of the hypothalamus to almost whole brain including the cerebellum. In the present study, using rat brain slice preparations and whole-cell patch recordings, the effects and ionic mechanisms of histamine on neurons in the cerebellar nuclei, the ultimate outputs of the cerebellum, were investigated. By means of electrophysiological and morphological identifications, two types of neurons in the cerebellar nuclei have been distinguished, i.e., type I neurons (projection neurons) and type II neurons (interneurons). Perfusing slices with 10, 30, 100 μM histamine only produced a dose-dependent excitatory response in type I neurons (n = 25) via postsynaptic H_2 receptors, but type II neurons had no response (n = 12). The histamine-induced excitation on type I neurons were found to be evoked by the depolarization of membrane potential (n = 9) under superfusing the slices with TTX. Furthermore, a simultaneous slight decrease in input resistance of the membrane (n = 8) indicated that one or more types of ion channels may be involved, and the histamine-induced depolarization was found to be blocked by ZD 7288 (n = 8). These results demonstrate that histamine selectively excites projection neurons rather than interneurons in the cerebellar nuclei through activation of the H_2 receptors and Ih channels. Considering our previous result that histamine improves rat motor balance and coordination via histamine H_2 receptors in the cerebellar interpositus nucleus (Song et al, Neuroscience, 2006), we suggest that the hypothalamocerebellar histaminergic fibers may modulate the final output of the cerebellum via their direct innervations on the nuclear projection neurons and may hold a key position in motor control.

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P51

Effect of Syntheetic Antioxidants on H2O2 Guinea-pig Colon Smooth Muscle Contraction (GPCC)

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Hydrogen peroxide (H_2O_2), one of the reactive oxygen species (ROS) is produced as a byproduct of many cellular reactions. It may regulate a variety of pathophysiological processes. We have shown that H_2O_2 contract ileum muscle and inhibits histamine release from RBL-2H3 cells [1,2].
We have now studied the effects of lipoic acid (natural antioxidant) and two newly synthesized derivatives ((UCLM084, a benzamide, and UCLM109, a hybrid molecule with the histone decacetylase inhibitor MGCDO1O3) on GPC contraction by H_2O_2, NaF (a non-specific G-protein activator) and carbachol (a muscarinic receptor agonist).
Experiments on GPCC were performed as previously described [2]. NaF, carbachol and H_2O_2 produced dose-related GPCC. Around 60, 60 and 50 % contractions above basal level were obtained with submaximal concentrations of NaF (10 mM), carbachol (0.05 μM) and H_2O_2 (1 μM), respectively. Each test compound (optimal concentration) was incubated together with one of the stimulants (submaximal concentration) for 15 min. MGCD0103 (1μM) produced 60 %, 60 %, 10 % inhibition against NaF, carbachol and H_2O_2 stimulation, whereas that for lipoic acid was 40, 35 and < 30 %, that for UCLM804 was 40, 36 and 31%, and that for UCLM109 was 50, 60 and 36%. The contractile effect of H_2O_2 is blocked by benzalkonium chloride (BCl), a G-protein inhibitor.
The present results that H_2O_2-induced contraction is inhibited by lipoic acid as well as UCLM084 and UCLM109, provides evidence that these compounds counteract the effect of oxidants/ROS on GPCC, and that a G-protein may be involved in their actions [3].

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P52

Histidine and Histamine Concentrations in Wines

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Different wines, particularly red wine, are beverages that contain significant amounts of histamine. Histamine is thought to be the main cause of adverse reactions to wines. In the case of ingestion of histamine, many of the same symptoms can appear without a true IgE allergic reaction taking place. Previously, it has been shown that wines (red, white wine, champagne) from different regions contain elevated concentrations of histamine. In this study, we investigated the concentrations of histidine and histamine in wine samples produced in Russia (Krasnodar region) and Ukraine (Krymea). The assessment was performed in 9 different non-bottled barrel wines (red, white wine, Champagne and Jerez) by amino acid assay and fluorimetry after a HPLC method, respectively. It has been shown that all wines contain different levels of histidine as well as histamine. The concentration of histidine elevated due to the time of fermentation and type of yeast. The highest concentration was determined in Jerez wines which elevated due to time after fermentation stopping. After 6 months the concentration of histidine in Jerez increased from 3 to 4-fold. The level of histamine in red wines (Cabernet and Merlo) was higher than in white wines (Chardonnay and Riesling). The concentration of histamine in two types of Jerez wines was extremely increased (up to 2.8-6.5 mg/ml) and that could be the consequence of its production by yeasts during continuous fermentation. The high concentration of histamine may explain the appetizing activity of Jerez wines. Despite elevated levels of histamine and histidine in some types of wines, in the following 1-month clinical study (with daily consumption of wines form 100 to 300 ml) we didn't find any clinical and immunological adverse effects of the wine samples with elevated histamine concentration.

P53

Feed Microscopy as a Possible Instrument n Allergy Prevention

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Feed control is an important element in the effort to guarantee consumer`s safety, especially food safety along the whole line from field to table.
The main instruments for feed control are chemical analysis to check feed additives, toxic substances such as heavy metals, mycotoxins or pesticides, pharmaceuticals like hormones or antibiotics, microbiological analysis to test the hygienic status of a sample or probiotics, molecular biology to find out if prohibited GMO (genetically modified organisms) have been used and feed microscopy.
The main functions of feed microscopy are the surveillance of the feed ban of animal meal for farm animals, to check if feed components are used as declared, but also to check for allergic risks, including infestations with moulds, insects and mites and contamination with toxic weed seeds or seeds of plants with allergic potential such as Ambrosia artemisiifolia.
The following study gives an overview over the last three years of microscopic feed control in Württemberg concerning the aspect of allergic risks for people dealing with feed in any way. It shows that feed microscopy can be one important tool in allergy prevention.

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Histamine restricted winemaking – from vintage to fining

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Individuals who suffer from histamine intolerance (HIT) often report that symptoms such as headache are caused or strengthened after drinking of wine, esp. red wine. The delight of wine consumption may be tempered by content of biogenic amines including histamine and tyramine. These natural occurring substances cause “pseudoallergic” reactions (1). High amine levels can make the beverage unfit for consumption due to toxicological aspects. Unfortunately, histamine and other amines cannot be tasted in wine. Histamine content of different wines is dependent of quality of grapes, wine manufacturing (hygienic and climatic conditions), microorganisms applied for malolactic fermentation (MLF) (2).
In our study, we present efficient methods for manufacturing of wines with low histamine concentrations. Prevention of microbial contamination starts in the wine yard by selection of immaculate grapes. In the wine cellar low temperature and pH (< 3.5), application of selected yeasts and lactobacilli without histidine decarboxlase (HDC) activity for alcoholic and malolactic fermentation, application of sulphur dioxide, proteolytic enzymes, fining and clarification agents after MLF are necessary criteria for histamine reduction. Histamine is cold- and heat-resistant and is difficult to remove additionally.
Until now the content of bioactive amines in wines in the EU is not legislatively regulated. Some countries have established limits for histamine in wines: Switzerland recommends 10 mg/L as maximum level, Germany 2 mg/L, Belgium 5 mg/L and France 8 mg/L.(3)
Guidelines for histamine restricted winemaking are helpful for the (hyper)sensitive consumer and prospective for compliance with legislation.

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P55

CIMETIDINE: A veteran H2-reseptor antagonist for the characterisation of novel potent ACYLGUANIDINE-type H2-receptor agonists

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During the development of novel potent acylguanidine-type histamine H2-receptor (H2R) agonists, we felt that it was obligatory to characterise the H2R-mediated nature of the positive chronotropic response elicited by these compounds in the classical guinea-pig assay (spontaneously beating right atrium) by using a specific H2R antagonist. Cimetidine, although being a veteran compound of medium affinity in this field [1], was chosen as the standard H2R antagonist due to its surmountable properties versus a large array of H2R agonists including histamine.
As expected, cimetidine (1.6 - 100 µM, incubation time 30 min) concentration-dependently shifted the concentration-effect curve (CEC) for histamine to the right. In the same fashion, CECs obtained for more than thirty novel acylguanidine-type H2R agonists were shifted in the presence of 10 µM cimetidine (higher antagonist concentrations were used in special cases). For selected H2R agonists, also increasing concentrations of cimetidine were titrated cumulatively into maximally stimulated atrial preparations. All experiments were performed in the presence of racemic propranolol (300 nM). The calculation of the affinity of cimetidine yielded pA2 values ranging from 5.7 up to 6.5, proving the H2R-mediated nature of the positive chronotropic effect of these acylguanidines.
However, in our hands, cimetidine and other typical H2R antagonists were surprisingly not able to antagonise the positive chronotropic response elicited by 3-(1H-imidazol-4-yl)propylguanidine (SK&F-91486 [2]), the long-known prototypic pharmacophore of H2R agonists of the guanidine (viz. impromidine) and the acylguanidine class of compounds. A similar behaviour was displayed by guanethidine, another guanidine-containing drug molecule with a second basic moiety.

[1] Brimblecombe RW et al, J Int Med Res 1975, 3, 86.
[2] Parsons ME et al, Agents Actions 1975, 5, 464.

P56

Dual histamine H1- and H4-receptor ligands

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In conclusion, our data provide important information about structural characteristics of ligands to act as antagonist or inverse agonist at hH4R. Thus, the results are a good starting point for developing dual H1R/H4R antagonists.
Histamine H1-receptor (H1R) antagonists are clinically important for treatment of allergic diseases, and the histamine H4-receptor (H4R) is discussed as a new therapeutic target for inflammation. The aim of this study was to analyze if H1R antagonists and agonists also show affinity to the H4R.
Thus, we coexpressed human (h) H1R and RGS4 on the one hand and hH4R-GAIP fusion protein with Gαi2 and Gβ1γ2 on the other hand in Sf9 insect cells and performed [3H]mepyramine- (hH1R) and [3H]histamine (hH4R) competition binding assays and steady-state GTPase assays. We characterized about 20 H1R antagonists and about 40 H1R agonists, like phenylhistamines and histaprodifens at hH1R and hH4R. In order to explain the pharmacological results on a molecular level, we constructed inactive and active models of hH1R and hH4R. The ligands were docked into the corresponding receptor models and molecular dynamic simulations, including the natural surrounding of the receptor were performed in order to obtain information about the binding modes.
For example, doxepine, a potent hH1R antagonist acts as an inverse agonist at hH4R, but compared to hH1R, the affinity is significantly decreased at hH4R. A comparable affinity at hH1R and hH4R was found for phenylhistamines with an additional histamine moiety and suprahistaprodifen. However, for most agonists, the affinity was higher at hH1R than at hH4R. By analogy to hH1R, phenylhistamines show partial agonism at the hH4R, but most histaprodifens, in contrast to hH1R act as inverse agonists at the hH4R. The modelling studies suggest that differences in amino acid sequence of transmembrane helices V and VI between hH1R and hH4R are responsible for the switch between agonism and inverse agonism between hH1R and hH4R.

P57

CNS histamine in rat model of vascular dementia

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[4] Department of Technology and Biotechnology of Drugs, Jagiellonian University, Medical College, Faculty of Pharmacy, Cracow, Poland

Background. Histaminergic system plays an important role in memory and learning. Deficient histaminergic transmission was suggested in the human brain in vascular dementia (VD) (Ishunina et al. 2004).
Aim. To get a better insight into the problem, a rat model of VD was used, based on permanent bilateral occlusion of the common carotid arteries (BCCAO), leading to chronic cerebral hypoperfusion.
Methods. Prior to the BCCAO, male Wistar rats underwent 7 days of training and only those animals that positively passed the holeboard memory test were elected for study. The operated rats were injected daily - for 6 days - with either monoamine oxidase B inhibitor, PF 9601N (40 mg/kg, i.p.), acetycholinesterase inhibitor, tacrine (3mg/kg, i.p), histamine H_3 receptor blocker, DL 76 (6 mg/kg, i.p) or saline. The first retest (R1) was performed one week after the surgery, while each subsequent test with a 5-7 day gap. The rats were sacrificed 2 or 4 weeks after the operation. Brain histamine (HA) concentration and the activity of histamine metabolising enzymes were measured with currently used procedures.
Results. BCCAO drastically increased latency and run time ($p<0.001$) 54±30 vs 3.4±1.2 and 268±18 vs 74 ±9, respectively, and affected rather working than reference memory, as measured by the 1st retest (R1). Treatment with either PF 9601N or tacrine seems to exert positive effect on working memory. This tendency disappeared after the drug treatment was stopped. Even if, latency and run time improved in R2 –R4, they have never attained the preoperative values.
Brain tissues from the rats, treated with PF 9601N, expressed only 15 % and 50% of untreated rat MAO B and MAO A activity, respectively, despite a 3 week discontinuation in the drug administration. Other examined drugs did not influence MAO enzymes. Histamine N-methyltransfrease activity did not show changes related to BCCAO or to the treatments.
Hypothalamic histamine concentration was significantly reduced after BCCAO 1.13±0.1 vs 1.91±0.16. Noteworthy, the rats, treated with PF 9601N or DL-76, do not reveal any significant differences, regarding their brain HA levels vs. their intact counterparts. The rat vascular dementia model supports the hypothesis of deficient histaminergic system in VD.
Ishunina TA, Kamphorst W, Swaab DF. J Neuropathol Exp Neurol. 2004; 63:1243-54

P58

The development of aniallergic and antihistaminic agents among polyphenols with high antiradical activity

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It is well-known that in allergic reactions the histamine liberation from the mast cells and the basophiles, depends on the permeability of their cytoplasmic and perigranular membranes. With raising the level of the peroxide oxidation of lipids, the products of peroxidation itself are capable of directly increasing the ionic permeability of lipid bilayer. The goal of this investigation was development of antiallergic and antihistaminic agents among the polyphenols with high antiradical activity.
Using quantum-mechanical analytical calculations of some derivatives of flavone and poly-hydroxychalcone we compared the antiradical activity to their antiallergic and antihistaminic activities using experimental in vivo methods of anaphylaxis (in mice) and in vitro (PBMC culture) models. It was shown that the derivatives of cinnamic acid manifest only antioxidant activity, whereas for the flavones and the chalcone both anti- and prooxidative activities The study of the different plant-derived flavonoids have been shown the existence of correlation between antiallergic and antihistaminic activities and their antiradical activity. Using methods of molecular construction, we realized the goal-directed synthesis of the significant group of the derivatives of 1,3-diazinon-4, some of which revealed the high antiallergic and antihistaminic activities in comparison to antiallergic compound ketotifen.

P59

Antiallergic and immunomodulating activity of N -[heteryl-substituted] of 4-[oxopyrimidine]

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The prospect of the search for new antiallergic, antihistaminic and immunotropic agents among the derivatives of 4-[oxopyrimidine] is explained by their structural similarity to the endogenous compounds. With the use of a logical structural approach to the formation of active immunotropic agents is realized the synthesis [heteryl-substituted] of 4-[oxopyrimidine], that contain different substitutes in the nucleoside position of heteronucleus. The primary pharmacological screening of the newly synthesized compounds showed that all realized structures (20 agents) inhibit some immune reactions (reaction of passive hemmagglutination and IgM-plaque-forming cells in mice)) from 50 to 100%. The majority of agents exceed Methyluracil's immunotropic activity. The presence of the of pyridine in the nucleoside position 4-oxo -1,4-[hydropyrimidine] has the much stronger stimulating effect on the cellular and humoral component of immune reaction, than the control antiallergic and antihistaminic compounds (ketotifen). Low toxicity in combination with the high antiallergic, antihistaminic and immunotropic activities suggest the possibility to further investigations of the derivatives of pyrimidine and/or quinazolinone as a potential classes of antiallergic substances.

P60

A novel series of potent histamineH_4 receptor antagonists: Orally bioavailable QUINAZOLINE SULFONAMIDES with anti-inflammatory activity *in vivo*

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The human histamine H_4-receptor (H_4R) is postulated to play a role in a variety of conditions including atopic asthma, itch and the modulation of pain .[1,2] In a scaffold hopping approach, a quinazoline was decorated with a variety of substituents[3], icluding several sulfonamide groups, leading to high H_4R affinity. Exploration of the SAR of the sulfonamide side chain revealed excellent tolerance to a range of different substituents, a property that is ideal for further optimization. The sulfonamides display a range of activities at the H_4R of rat, mouse and human ranging from agonism to antagonism and inverse agonism. In addition. *in-vivo* administration of these orally bioavailable compounds to rats revealed them to have potent anti-inflammatory effects.

[1] Oda, T., et al. *J. Biol. Chem.* **2000**, 275:36781-36786
[2] Lim, H., et al. *Curr. Top. Med. Chem.* **2006**, 6:1365-1373
[3] Smits, R., et al. *J. Med. Chem.* **2006** *49*, 4512-4516.

P61

Computational Approach to Structure, Function and Specific Intervention on Mammalian Histidine Decarboxylase

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Histidine decarboxylase (HDC, EC 4.1.1.22), the enzyme responsible of histamine synthesis, has been long pointed out as a main target for developing new inhibitors of its activity, in order to contain the overproduction of histamine under pathological conditions. This approach is an alternative to the actual antihistaminic drugs which are focused to avoid the binding between histamine and any of its four known receptors.
Due to its instability, HDC structure has not been solved by any experimental approach yet. Our group is focused on the structural and functional characterization of HDC using a computational approach. First a homology model of mammalian HDC structure was generated and validated by directed mutagenesis experiments [1]. Secondly, decarboxylation reaction was studied by means of QM/MM simulations. The results obtained were in accordance with the experimental results obtained in our laboratory [2]. In our search for new potential inhibitors of HDC activity we have explored the conformational landscape of the molecule. Two systems have been studied, simulating both internal and external aldimine conformations of the active site, and the principal movements were observed. Among them, special attention was paid to a loop (residues 331 to 349), which has been proposed to close on the active site entrance once the ligand has been bound to the enzyme, and it is a shared structural features in several PLP-dependent decarboxylases.
These structural and functional features observed enabled us to develop a virtual screening project [3] in which a database comprising 150 molecules, analogs of the substrate histidine, were docked in HDC active site and evaluated as new potential inhibitors of mammalian HDC. In this communication we offer an overview of all our computational study and focus on our new virtual screening results.

[1] Moya-Garcia, A.A., M.A. Medina, and F. Sanchez-Jimenez, Mammalian histidine decarboxylase: from structure to function. Bioessays, 2005. 27(1): p. 57-63.
[2] Moya-Garcia, A.A., et al., Analysis of the decarboxylation step in mammalian histidine decarboxylase. A computational study. J Biol Chem, 2008. 283(18): p. 12393-401.
[3] Moya-García AA, Pino-Ángeles A, Gil-Redondo R, Morreale A, Sánchez-Jiménez F. Structural Features of Mammalian Histidine Decarboxylase Reveal the Basis for Specific Inhibition. Br J Pharmacol. (In press)

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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.

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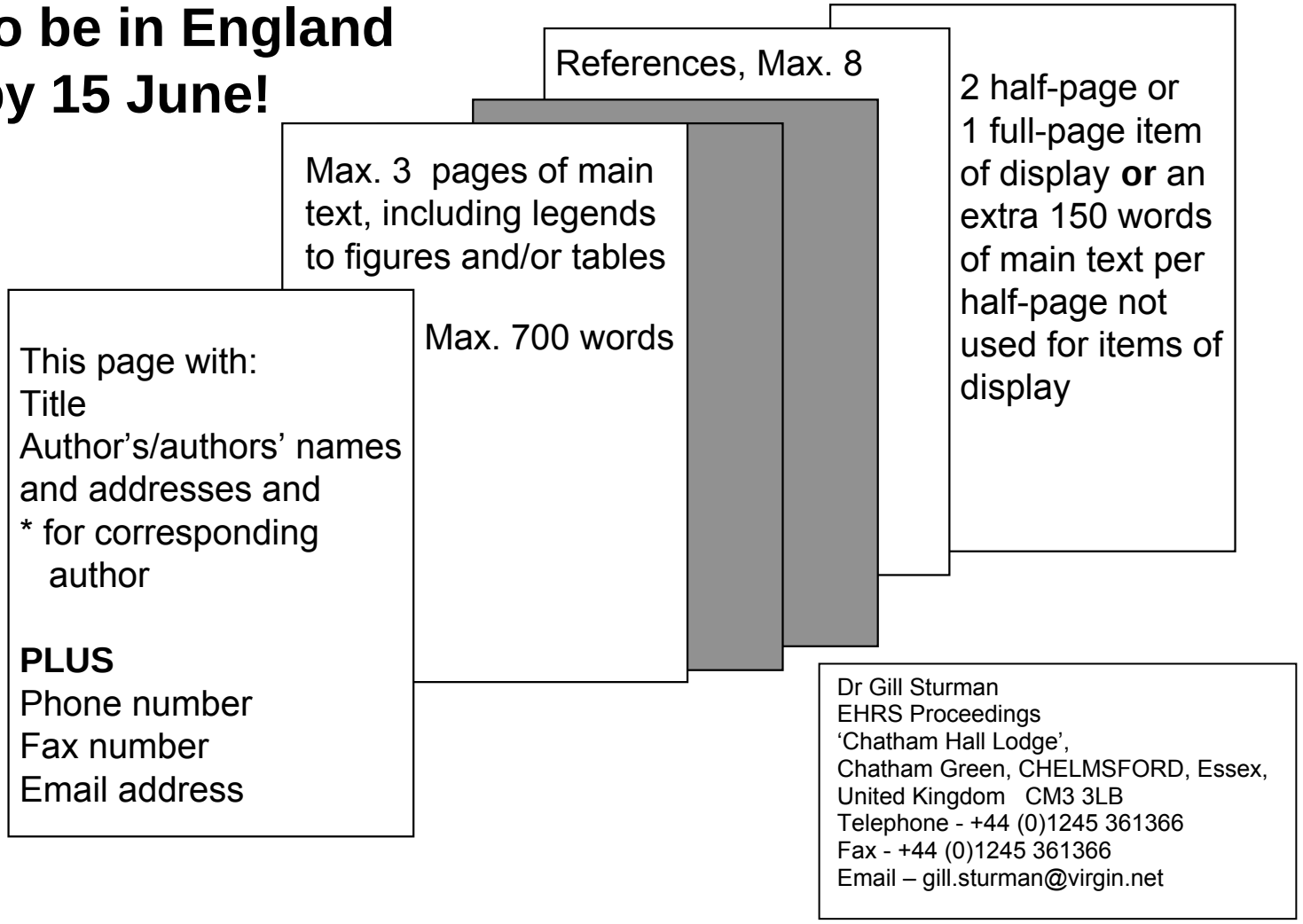

THE INTERNATIONAL ANTHEM OF THE EUROPEAN HISTAMINE RESEARCH SOCIETY

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 your 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 / it was also very 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 is bad.
The 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!
Back to Stockholm that we knew / with a lovely water view
With Anita in the North two thousand eight.

26. Then to **Fulda** the next year / we´re in Germany to hear
How our Frido with Histamine can play.
Let us sing this song together / Histamine will last forever
Singing Histaminosis all the day.

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