European Histamine Research Society

XXXVIIth Meeting

Stockholm, Sweden 7th – 10th May 2008

PROGRAMME and ABSTRACTS

Organizer: Centre for Allergy Research, Karolinska Institutet

Congress Venue: Skogshem & Wijk Conference Center, Lidingö

Contents

Sponsors

The Arthur A Hancock Young Investigator Award

Abbott
Mrs Kathryn Hancock

Student Travel Bursaries

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Previous EHRS Annual Meetings

1970's	1980's	1990's	2000's
1971 Lodz	1980 Visegrad	1990 Kuopio	2000 Nemi (Rome)
1972 Paris	1981 Hannover	1991 Marburg	2001 Turku
1973 Marburg	1982 Bled	1992 Malaga	2002 Eger
1974 Copenhagen	1983 Brighton	1993 Cologne	2003 Noordwijkerhout
1975 Florence	1984 Florence	1994 Budapest	2004 Bergisch-Gladbach
1976 Paris	1985 Aachen	1995 Moscow	2005 Bled
1977 London	1986 Odense	1996 Antwerp	2006 Delphi
1978 Lodz	1987 Strbske Pleso	1997 Seville	2007 Florence
1979 Stockholm	1988 Copenhagen	1998 Lodz	
	1989 Breda	1999 Lyon	

Welcome

Dear Histaminologists,

We are happy to welcome you back to Stockholm for the XXXVIIth EHRS meeting, 7th to 10th May 2008. The congress venue is in Lidingö, an Island only 20 min from Stockholm city centre. Stockholm is the capital of Sweden with population of about 1.5 million, including the suburbs. Stockholm is a very beautiful city built on 14 islands where Lake Mälaren flows out into the Baltic Sea and the Stockholm Archipelago, a unique maritime landscape of more than 24,000 islands.
Stockholm with all its islands and waterways has also been called the Venice of the North. In the centre are Gamla Stan (the Old Town) and Riddarholmen, two islands that together make up northern Europe´s largest and best preserved medieval city, with a history dating back to the 13th century. Although Stockholm is the financial and political centre of Sweden, it is best known for its reputation in Fine Arts and Science and the Medical Faculty at the Karolinska Institutet awards the Annual Nobel Prize in Physiology and Medicine. The Nobel Museum in Stockholm can be visited to learn about the great scientists and poets who have been laureates in the past. There are also many other museums, exhibitions and cultural events that can be visited, for example the famous Skansen outdoor museum of Swedish heritage or the Vasa ship dating from the 17th century. You can take a boat from the town centre out to the Archipelago, or go fishing for fresh salmon right in the city centre close to the Royal Palace.
The programme for this year's meeting is very exciting with seven special lectures, 33 oral presentations and 40 poster presentations. The GB West lecture will be given by John Bienenstock and Plenary lectures by Pertti Panula and Kristoffer Hellstand. There will be a special Mast Cell Symposium with a prominant faculty. On the last day we have the Arthur A Hancock Young Investigator Award Symposium with the presentations by the five finalists for the precious Award sponsored by Abbott and Mrs Kathryn Hancock.
On behalf of the organizing committee we wish you heartily welcome!

Sven-Erik Dahlén, Meeting Chairman

Anita Sydbom, Meeting Co-Chairman, President EHRS

Committees

Organizing Committee

Staffan Ahlstedt
Sven- Erik Dahlén
Madeleine Ennis
Gunnar Nilsson
Yvonne Nygren
Anita Sydbom

Abstract Evaluation Committee

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Patrizio Blandina
Paul Chazot
Friedhelm Diel
Bernie Gibbs
Gill Sturman
Ekaterini Tiligada
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The Arthur A Hancock Young Investigator Award Committee

Paul Chazot (chairman)
John Bienenstock
Marlon Cowart

Poster Prize Committee

Friedhelm Diel (chairman), Mikael Adner, Frank Ahrens, El-Sayed Assem, Agniezska Fogel, Tatjana Irman-Florjanc, Elena Rivera, Walter Schunack

General Information

Host Institute
Centre for Allergy Research, Karolinska Institutet
P O Box 287, SE-171 77 Stockholm, Sweden

Congress Venue
Skogshem & Wijk Conference Center, Lidingö, Sweden

Registration Desk
The registration desk at the Wijk hotel entrance hall will be open:

Wednesday	7th May	15.00 -18.00
Thursday	8th May	08.00 -18.00
Friday	9th May	08.00 -12.00
Saturday	10th May	08.00 -12.00

Conference Website and E-mail/ Internet Service
The conference website adress is http://ki.se/EHRS2008 and the EHRS website address is www.EHRS.org.uk. E-mail and Internet Service is provided by Skogshem & Wijk. PCs with internet connection are found by the Wijk main reception. If you have your own wireless enabled laptop PC you can request log-on information to our wireless network at the reception.

Oral Communications
Oral presentations should last no longer than 10 min and there will be 5 min for discussion. The presentations should be in MS Power Point format. 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 drive, saved in a file as: Name_of_presenter.ppt

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. We encourage the presenters to stand by their posters during the coffee breaks and the poster session.
The maximum poster size is 100 x 70 cm (h x w). All posters should have their corresponding code consisting of the letter "P" and a two-digit number (P__) in an A5 sized area (140 x 210 mm; h x w) on the top left corner of your poster. The code font should be Arial bold (or equal) and the size 240 pt (about 6 cm). Material to attach the posters will be provided. Posters which have made the final short-listing for the poster prizes will be revisited by the Poster Jury on Saturday afternoon. Prizes will be announced at the Farewell Dinner.

Social Programme

Wednesday	**7th May**	18.30 Reception and Welcome Party With Buffet Dinner At the Congress Centre
Thursday	**8th May**	19.00 Dinner with Entertainment – A Swedish Evening At the Congress Centre
Friday	**9th May**	13.30 Excursion with boat to Stockholm Town (dress: comfortable shoes) The Wasa Museum Town Ferry to the Old Town Walking through the Old Town The Nobel Museum Late buffet Dinner at the Nobel Museum 22.30 Bus back to Skogshem & Wijk
Saturday	**10th May**	19.30 Farewell Dinner with music and dancing (dress: lounge suit/elegant dress recommended but not essential)

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Accompanying Partners Programme

A special Programme for Accompanying Partners will be provided

The Programme at a Glance

Wednesday May 7th 2008

15.00- Arrival – Check in - Registration
16.00-18.00 EHRS Council meeting
18.30- Reception - Welcome party

Thursday May 8th 2008

08.30- 09.30 Opening Session
09.30-10.30 Plenary Lecture: Kristoffer Hellstrand, Sweden
Post-consolidation therapy with histamine dihydrochloride and interleukin-2 in acute myelogenous leukemia.
10.30-11.00 Coffee break and poster viewing
11.00-12.00 Oral session I: *Histamine and Neurobiology*
12.00-13.00 Oral session II: *Histamine and Neurobiology II*
Group Photo Session
13.00-14.00 Lunch
14.00-14.30 Lecture: Sven Erik Dahlén, Sweden
"Prostaglandin D_2: Mediator of inflammation and sleep, as well as sensitive in vivo marker of mast cell activation"
14.30-15.30 Oral session III: *H_4 Receptors*
15.30-18.30 Coffee break and Poster Session
16.00-17.15 Poster session 1 and 3 in parallel
17.15-18.30 Poster session 2 and 4 in parallel
19.00- Dinner - Entertainment

Friday May 9th 2008

08.30-9.30 G.B.West Lecture: John Bienenstock, Canada
Mast cells, nerves and IgE
09.30-11.00 *Mast cell symposium*
11.00-11.30 Coffee break and poster viewing
11.30-13.00 Oral session IV: *Mast cells and Histamine release*
13.30-23.00 Excursion with boat trip starting with lunch on board

Saturday May 10th 2008

08.30-09.30 Plenary Lecture: Pertti Panula, Finland
The vertebrate brain histaminergic system: a multifaceted modulatory network.
09.30-11.15 Oral session VI: *Histamine potpourri*
11.15-11.45 Coffee break and poster viewing
11.45-13.00 *Art A Hancock Young Investigator Award Symposium*
13.00-14.00 Lunch
14.00-15.00 Oral session VII: *Histamine the Future*
14.30-15.00 Sven-Erik Dahlén
New EU Funding Opportunity: Innovative Medicines Initiative – IMI
15.00-16.00 Coffee Break and Poster Final
16.00-18.00 General Assembly Meeting
19.30- Farewell Dinner

Sunday May 11th 2008

Check out – Departure

Scientific Programme

Wednesday May 7th

15.00- **Registration**
16.00-18.00 **EHRS Council meeting**
18.30 **Reception**
Welcome party

Thursday May 8th

08.30- 09.30 **Opening Session**
Sven-Erik Dahlén **Chairman of the Meeting**
Anita Sydbom **President of EHRS**
Distribution of Bursaries

09.30-10.30 **Plenary Lecture**
L7 **Kristoffer Hellstrand, Gothenburg, Sweden**
Post-consolidation therapy with histamine dihydrochloride and interleukin-2 in acute myelogenous leukemia.
Introduced by: Gunnar Nilsson

10.30-11.00 Coffee break and poster viewing

11.00-12.00 **Oral session I: *Histamine and Neurobiology***
Chaired by: Patrizio Blandina and Pertti Panula

11.00.11.15
O1 **Rob Leurs, Kathleen M. Krueger, Jose-Antonio Arias-Montano, Thomas R. Miller, John L. Baranowski, Brian R. Estvander, David G. Witte, Marina I. Strakhova, Remko A. Bakker, Marlon D. Cowart, Timothy A. Esbenshade and Gerold Bongers**
Distinct signaling properties of human H_3 receptor isoforms.

11.15-11.30
O2 **Chiara Mariottini, Tania Scartabelli, Patrizio Blandina, Domenico Pellegrini-Giampietro and Maria Beatrice Passani.**
H_3 receptor agonists activate antiapoptotic pathways in rat cortical neurons and protect against neurotoxic insults.

11.30-11.45
O3 **Saara Nuutinen, Kaj Karlstedt and Pertti Panula**
The Effects of Ethanol on the Regulation of Motor Behaviour in HDC Knockout Mice.

11.45-12.00
O4 **Samotaeva Irina. S., Kuznetsova G.D. and Tuomisto L.**
Immediate and delayed effects of metoprine injection in WAG/Rij rats.

12.00-13.00 **Oral session II:** ***Histamine and Neurobiology II***
Chaired by: Beatrice Passani and Marija Čarman-Kržan

12.00-12.15
O5 **Patrizia Giannoni, Maria Beatrice Passani, Leonardo Munari, Renato Corradetti and Patrizio Blandina**
Citalopram, a selective serotonin reuptake inhibitor (SSRI), increases histamine release in the brain through its effect on endogenous 5-HT

12.15-12.30
O6 **Paul L Chazot, William Connelly, Fiona C Shenton, Natasha Lethbridge, Ruan van Rensburg and George Lees**
Further Evidence for H_4 Histamine Receptor Expression on Central Neurons.

12.30-12.45
O7 **Nicholas P. Clarke , Christopher D. Brown, Charlotte Lane, Charlie Mowbray, Herman D. Lim, Rob Leurs, Emanuel Schenck, Christelle Perros-Huguet and Michael Yeadon**
PF-2988403 – an 'H4 antagonist' demonstrating the full range of in vitro pharmacologies which translate in vivo in the rat.

12.45-13.00
O8 **Simona Rajtar and Tatjana Irman-Florjanc**
Could Antidepressants Noticeably Affect Histamine Degrading Enzymes and Consequentially Inflammatory Processes? A Study in Guinea Pig and Rat.

13.00-14.00 Group Photo Session and Lunch

14.00-14.30
L2 **Sven Erik Dahlén, Sweden**
"Prostaglandin D_2: Mediator of inflammation and sleep, as well as sensitive in vivo marker of mast cell activation"

14.30-15.30 **Oral session III:** ***H_4 Receptors***
Chaired by: Nick Carruthers and Andras Falus

14.30-14-45
O9 **R. Leurs, R.A. Smits, H. Lim, and M. Adami, G. Coruzzi and I. de Esch**
Probing the histamine H_4 receptor binding site: from fragments to active ligands.

14.45-15.00
O10 **E. Masini, M.C. Vinci, S. Nistri, L. Cinci, R. Mastroianni, R. L Thurmond and D. Salvemini.**
A selective H_4R antagonist prevents antigen-induced asthma-like reaction and airway inflammation in guinea pigs.

15.00-15.15
Oral (P40) **Nicholas I Carruthers**
Histamine H3 Receptor Antagonists: Correlation of Pharmacokinetic and Pharmacodynamic Effects with Physical Properties.

15.15-15.30
O12 **Peter Pócza Zoltan Wiener, Gergely Tolgyesi, Zoltan Pos, Viktor Molnár, Zsuzsa Darvas, Kristof Dede and Tamas Mersich, *Zsolt Baranyai, Ferenc Jakab, Andras Falus***
Microarray profiling and pathway analysis of colorectal cancer.

15.30-18.30 Coffee break and Poster Session
Coffee will be available from 15.30 and also pre dinner drinks

16.00-17.15 **Poster sessions 1 and 3 in parallel**

16.00-17.15 **Poster session 1: *Histamine Receptors***
Chaired by: Mikael Adner and Frank Ahrens

P1 **Rado Nosáľ, Viera Jančinová, Viera Nosáľová, Tomáš Perečko, Milan Číž and Antonín Lojek**
Pheniramines and Oxidative Burst of Blood Phagocytes During Ischaemia/Reperfusion

P2 **Viera Nosáľová, Katarína Drábiková, Viera Jančinová, Radomír Nosáľ, Tatiana Mačičková, Jana Pečivová, Jana Nedelčevová and Ružena Sotníková**
Protective effect of Pheniramines against mesenteric ischaemia/reperfusion-induced injury.

P3 **Frank Ahrens, Yvonne Sünkel, Tanja Pollmüller, Ralf Bussemas, Friedrich Weissmann and Michael H. Erhard.**
Plasma Histamine-Levels in Organic Farming Piglets: Differences Due To Immunization and Weaning.

P4 **Kristine Roßbach, Stefanie Wendorff, Kerstin Sander, Holger Stark, Manfred Kietzmann and Wolfgang Bäumer**
The selective histamine H4 receptor antagonist JNJ7777120 exhibit antipruritic efficacy in two murine models of contact dermatitis, but does not affect inflammation.

P5 **Susanne Mommert, Gitta Köther, Katja Zwingmann, Thomas Werfel and Ralf Gutzmer**
Expression and function of histamine H_4 receptor in human $CD4^+$ T cells.

P6 **E Sakurai, A Kuramasu, N Okamura, T Watanabe and K Yanai**
Histamine receptors genes knockout mice and their phenotypes.

P7 **Natalia C. Fernandez, Federico Monczor, Cintia Notcovich, Federico Gottardo, Carina Shayo and Carlos Davio**
Trafficking of Histamine H2 Receptor, Phosphorylation Dependent and Independent Regulation of H2r by GRK2.

P8 **Natasha Lethbridge, Fiona C. Shenton, Victoria Hann and Paul L. Chazot**
The First Panel of Immunological Probes for Human H_3 Histamine Receptor Isoforms.

P9 **Lovro Ziberna, Tadej Zorman and Gorazd Drevenšek**
Characterization of Histamine-Induced Relaxation in Pre-Contracted Rat Aorta.

P10 **Špela Sprogar, Andrej Cör, Martina Drevenšek and Gorazd Drevenšek**
Famotidine Increases Bone Volume in the Late Stage of Orthodontic Tooth Movements in Rats.

P11 **Danuta Maslinska, Milena Laure-Kaminowska, Krzysztof T Maslinski, Elzbieta Wojtecka-Łukasik, Dariusz Szukiewicz and Slawomir Maslinski.**
Histamine-releasing factor in human choroid plexus.

16.00-17.15 **Poster session 3: *Histamine, Allergy and Inflammation***
Chaired by: El-Sayed K Assem and Tatjana Irman-Florjanc

P21 **Parmjeet S. Panesar, Shogofa Ahad and Bernhard F. Gibbs**
Differential effects of potassium channel blockers on anti-IgE-stimulated histamine release from human basophils.

P22 **Katarina Černe and Mojca Kržan**
Involvement of Organic Cation Transporter in Histamine Uptake into Human Vascular Endothelial Cells.

P23 **Maria Herwald, Hannelore Borck, Susanne Diel and Friedhelm Diel**
Histamine in Beer and "beer allergies".

P24 **Jean Sainte-Laudy**
Differential effect of storage on histamine molecular and ultra-molecular dilutions.

P25 **Jean Sainte-Laudy**
Interest of the measurement of basophil's reactivity and sensitivity for pharmacology and allergy diagnosis.

P26 **Rob Leurs, Rogier Smits, Martien Mooijer, Iwan de Esch and Bert Windhorst.**
Synthesis and in-vivo evaluation of human histamine H_4 receptor modulators [^{11}C]JNJ7777120 and [^{11}C]VUF10558 for monitoring inflammatory processes using PET.

P27 **Jesper Säfholm, Lars-Olaf Cardell, Sven-Erik Dahlén and Mikael Adner**
Cyclooxygenase Metabolites Reduce Airway Smooth Muscle Response to Histamine in Guinea-pig Trachea.

P28 **Hang Yung Alaster Lau and Ka Yan Jessica Law**
Effects of Oestrogenic Agents on Rat Peritoneal Mast Cells.

P29 **Flora Gaber, Kameran Daham, Ai Higashi, Noritaka Higashi, Agneta Gulich, Ingrid Delin, Anna James, Nurdan Sandalci, Pär Gyllfors, Maria Skedinger, Sven-Erik Dahlén, Maria Kumlin and Barbro Dahlén**
Increased Levels of Cysteinyl-Leukotrienes in Blood, Urine, Saliva and Induced Sputum from Aspirin-Intolerant Asthmatics.

17.15-18.30 **Poster sessions 2 and 4 in parallel**

17.15-18.30 **Poster session 2: *Histamine, CNS and Cardiovascular Regulation***
Chaired by: Agnieszka Fogel and Walter Schunack

P12 **Eiichi Sakurai , Eiko Sakurai, T. Watanabe and K. Yanai**
Uptake of L-histidine and histamine biosynthesis at the blood-brain barrier.

P13 **P. Van Ruitenbeek, A. Vermeeren, F. Smulders, A. Sambeth and W. J. Riedel.**
Selective effects of dexchlorpheniramine on stages of human information processing.

P14 **Katja Perdan , Zala Kobe, Veronika Kralj- Iglič and Mojca Kržan**
Nature of histamine transport in neonatal rat cultured astrocytes.

P15 **Matthias Nettekoven, Jean-Marc Plancher, Susanne Raab, Hans Richter, Olivier Roche, Rosa María Rodriguez Sarmiento, Sven Taylor, Christoph Ullmer and Atsushi Yamatodani**
Withdrawn

P16 **Maristella Adami, Cristina Pozzoli, Gabriella Coruzzi, Rogier A. Smits and Rob Leurs**
Histamine H4 receptors are not involved in the regulation of cholinergic neurotransmission in the rat duodenum.

P17 **Richard Andersson, Andre Fisahn and Maria Lindskog**
H3 receptor stimulation decreases gamma oscillations in the hippocampus.

P18 **Jerzy Jochem**
Orexin type 1 receptor antagonist SB 334867 inhibits central histamine-induced resuscitating effect in rats subjected to haemorrhagic shock.

P19 **Jerzy Jochem, Agata Niwecka, Ewa Olearska and Tatjana Irman-Florjanc**
Interactions between the serotonergic and histaminergic systems in the central cardiovascular regulation in haemorrhage-shocked rats: involvement of 5-HT1A receptors.

P20 **Marija Čarman-Kržan and Damijana M. Jurič**
Withdrawn

17.15-18.30 **Poster session 4:** ***Histamine Metabolism, Inflammation and Tumour Growth***

Chaired by: Friedhelm Diel and Elena Rivera

P30 **Fernanda Genre, Eduardo Valli, Vanina A. Medina, Alicia S. Gutiérrez, Lorena A. Sambuco, Elena S. Rivera, Graciela P. Cricco and Gabriela A. Martín**
Effect of histamine on metalloproteinases expression and cell adhesion in breast cancer cell lines.

P31 **Konstantinos Papamichael, Evangelia Zampeli,Gerasimos J Mantzaris and Ekaterini Tiligada**
Blood Histamine Levels in Patients with Inflammatory Bowel Disease on Infliximab Therapy: Preliminary Results.

P32 **Konstantinos Kyriakidis, Evangelia Zampeli and Ekaterini Tiligada**
A Pilot Study on Peripheral Blood Histamine Levels in Women with Ductal Breast Cancer.

P33 **Cintia Notcovich, Federico Diez, Sabrina Copsel, Marcelo Kazanietz, Carlos Davio and Carina Shayo**
Stimulation of H1 Receptor Inhibits Cell Proliferation through Activation of Small G Proteins in CHO-H1 Cells.

P34 **Michael A. Kuefner, Johannes Feurle, Michael Uder, Werner Bautz and Hubert G. Schwelberger**
The effect of iodinated and gadolinium contrast media on the histamine degrading enzymes diamine oxidase and histamine methyltransferase – an in-vitro study.

P35 **E.Wojtecka-Lukasik, P. Rzodkiewicz, D. Maslinska, D. Szukiewicz, W. Schunack and S. Maslinski**
Histamine chloramine modifies casein-induced inflammation.

P36 **Dariusz Szukiewicz , Grzegorz Szewczyk, Michal Pyzlak, Aleksandra Stangret and Danuta Maslinska**
Increased expression of histamine H1 receptor by human amniotic epithelial cells (HAEC) in chorioamnionitis (CHA) is accompanied by augmented production of secretory leukocyte protease inhibitor (SLPI).

P37 **Dariusz Szukiewicz , Jakub Klimkiewicz , Michal Pyzlak, Jerzy Stelmachow and Danuta Maslinska**
Increased permeability of human amnion to calcium ions in chorioamnionitis (CHA) is related to histamine H1 receptor overexpression within amniotic epithelial cells (AEC).

P38 **Vanina A Medina, Noelia A Massari, Mariel A Núñez, Graciela P Cricco, Gabriela A Martín, Rosa M Bergoc and Elena S Rivera.**
Inhibits Proliferation through the Four Histamine Receptor Subtypes in MCF-7 Human Breast Cancer Cell Line.

P39 **Simona Rajtar, Katarina Černe, Mojca Kržan and Tatjana Irman-Florjanc**
The Activity Of Histamine Degrading Enzymes And Uptake Of Histamine In Human Vascular Endothelial Cells: Influence Of Antidepressants

19.00 **Dinner**
Entertainment

Friday May 9th

08.30-09.30 **G.B.West Lecture**
L3 **John Bienenstock, Canada**
Mast cells, nerves and IgE
Introduced by: Sven- Erik Dahlén

09.30-11.00 ***Mast cell symposium***
Chaired by: Madeleine Ennis and Staffan Ahlstedt

09.30-10.00
L4 **Gunnar Nilsson, Sweden**
Differential release of mast cell mediators - implications in health and disease.

10.00-10.30
L5 **Gunnar Pejler, Sweden**
The role of serglycin proteoglycan in mast cell secretory granule formation

10.30.11.00
L6 **Ulrich Blank, France**
Intracellular trafficking and signaling mechanisms in the secretion of inflammatory mediators and histamine by mast cells.

11.00-11.30 Coffee break and poster viewing

11.30-13.00 **Oral session IV:** ***Mast cells and Histamine Release***
Chaired by: Bernie Gibbs and Alaster Lau

11.30-11.45
O13 **Magdalena Gulliksson and Gunnar Nilsson**
Mast cell degranulation and mediator secretion in response to hypoxia - implications in host defence.

11.45-12.00
O14 **Kwok Ho YIP, Lai Lok WONG and Hang Yung Alaster LAU**
Adenosine: Role of Different Receptor Subtypes in Mediating Histamine Release from Human and Rodent Mast Cells.

12.00-12.15
O15 **Shogofa Ahad, Parmjeet S. Panesar, Martin K. Church and Bernhard F. Gibbs**
Effects of varying pH on IgE-dependent histamine secretion from human basophils.

12.15-12.30
O16 **Hubert G. Schwelberger**
Histamine intolerance: overestimated or underestimated?

12.30-12.45
O17 **Beatrice Y Wan, Kheng H Peh, El-Sayed K Assem, Brian Middleton, Jon Dines and Charles M Marson**
Effects of histone deacetylase inhibitors on rat mast cell activation and rat colonic smooth muscle.

12.45-13.00
O18 **Egle Passante, Carsten Ehrhardt, Helen Sheridan and Neil Frankish.**
Toll-like receptors and RBL-2H3 mast cells.

13.30-23.00 **Excursion with boat trip starting with lunch on board**

Saturday May 10th

08.30-09.30 **Plenary Lecture**

L1 **Pertti Panula, Finland**
The vertebrate brain histaminergic system: a multifaceted modulatory network.
Introduced by: Rob Leurs

9.30-11.15 **Oral session VI: *Histamine Potpourri***
Chaired by: Piero Mannaioni and Gill Sturman

9.30-9.45
O24 **Vanina A Medina , Maximo Croci, Nora A Mohamad, Ernesto JV Crescenti, Rosa M Bergoc and Elena S Rivera.**
Subcellular Localization of Histamine H3 Receptor in Human Mammary Cells.

9.45-10.00
O25 **Evangelia Zampeli, Pothitos Pitychoutis, Zeta Papadopoulou-Daifoti and Ekaterini Tiligada**
Systemic Challenge with Lipopolysaccharide Induces Histamine Levels in the Rat Conjunctiva and Cartilage but not in the Hypothalamus.

10.00-10.15
O26 **Jutta Rosner , Cathleen Krieg, Inna Michel, Nadja Schulz, Natalia Kuzmenko, Heike Weisser and Friedhelm Diel**
Th17 in sensitized human lymphocytes ex vivo.

10.15-10.30
O27 **El-Sayed K Assem, Kheng H Peh, Beatrice Y Wan, Brian Middleton, Jon Dines and Charles M Marson**
Effects of a selection of histone deacetylase inhibitors on antigen- and agonist-induced airway smooth muscle contraction

10.30-10.45
O28 **Jerzy Jochem, W. Agnieszka Fogel, Barbara Rybus-Kalinowska, Murat Yalcin and Vahide Savci**
Involvement of the histaminergic system in cytidine 5'-diphosphocholine-induced reversal of critical haemorrhagic hypotension in rats.

10.45-11.00
O29 **Kamil J. Kuder, Xavier Ligneau, Jean-Claude Camelin, Jean-Charles Schwartz, Holger Stark, Walter Schunack, Dorota Łażewska and Katarzyna Kieć- Kononowicz**
Diether (substituted)piperidine derivatives as a novel, active histamine H_3 receptor ligands.

11.00-11.15
O30 **Anna-Karin Larsson, and Sven-Erik Dahlén.**
Effective beta adrenergic inhibition of mast cell mediator release in the peripheral lung.

11.15-11.45 Coffee break and poster viewing

11.45-13.00 ***Arthur A Hancock Young Investigator Award Symposium***

Chaired by: Paul Chazot and Marlon Cowart

11.45-12.00
O19 **Zoltan Wiener, Melinda Racz and András Falus**
IL-18 stimulates Ccl1 expression in mouse non-activated mucosal mast cells.

12.00-12.15
O20 **Kamonchanok Sansuk, R.A. Bakker, Anne Watts, L. Pardo and R. Leurs**
Residue 6.45 in the histamine H_1 receptor acts as a switch for ligand-mediated activation.

12.15-12.30
O21 **Agnes Koncz , Maria Pasztoi, Mercedesz Mazan, Edit Buzas, Gyorgy Nagy and András Falus**
Altered T Cell Nitric Oxide Production and Cytokine Production in Histidine Decarboxylase Knockout Mice.

12.30-12.45
O22 **Rajkumar Noubade, Naresha Saligrama, Karen Spach, Roxana del Rio, Elizabeth P. Blankenhorn, Theodoros Kantidakis, Graeme Milligan, Mercedes Rincon and Cory Teuscher**
Polymorphisms in Murine Histamine Receptor H_1 Lead to Differential Cell Surface Expression and Influence Autoimmune Disease Progression.

12.45-13.00
O23 **Kristoffer Sahlholm, Johanna Nilsson, Daniel Marcellino, Kjell Fuxe and Peter Århem**
Differential Voltage-Sensitivity of Human Histamine H_3 and H_4 Receptors.

13.00-14.00 Lunch

14.00-15.00 **Oral session VII: *Histamine the Future***
Chaired by: Hubert Schwelberger and Ekaterini Tiligada

14.00-14.15
O31 **Maria Gschwandtner, Rahul Purwar, Miriam Wittmann, Wolfgang Bäumer, Manfred Kietzmann, Thomas Werfel and Ralf Gutzmer**
Histamine H_1 receptor stimulation on keratinocytes leads to increased MMP-9 production.

14.15-14.30
O32 **Viktor Molnár, Hargita Hegyesi, Viola Tamási, Sára Tóth, Zoltán Wiener and András Falus**
Mast cell-derived histamine: in vivo and in vitro effects on tumor growth.

14.30-15.00
O33 **Sven-Erik Dahlén**
IMI – a new opportunity for research funding in Europe

15.00-16.00 Coffee Break + Poster Final

16.00-18.00 **General Assembly**

19.30- **Farewell Dinner**

Invited Lectures

L 1

The Vertebrate Brain Histaminergic System, A Multifaceted Modulatory Network

Pertti Panula
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Histamine has emerged as a characteristic modulatory neurotransmitter in vertebrate brain. Physiological functions are performed by complex networks, and the role of histamine and individual histamine receptors is difficult to reveal. Histamine is involved in e.g. alerteness and sleep, memory, feeding and drinking, hormone regulation, memory, anesthesia, and hibernation.
Although the basic organization of the histaminergic system, there are also interesting differences. The transient fetal rhombencephalic histamine system is also limited to some vertebrates, including mammals, and its functions are still largely unknown. The putative role of this system in development of neurons and neural stem cells is intriguing and needs more experimental evidence. Despite the differences in developmental histamine, all four G-protein coupled histamine receptors are expressed in vertebrate brain, but the expression patterns are different. The lack of histamine in some brain areas with high expression of histamine receptors renders it possible that there are still unknown functional aspects. Since histamine receptors are also found in endothelial and ependymal cells and astrocytes in addition to neurons, diurnal regulation of brain histamine may be important for several functions.
Since histamine levels decrease in Alzheimer's disease, some cognitive problems may be due to functional failures of the histaminergic neurons. This is supported by interactions of histamine, GABA and acetylcholine in regulation of the septohippocampal pathway. In Parkinson's disease, evidence from human and experimental animal studies renders it possible that the histaminergic system is important in disease progress.
Although lack of brain histamine is not fatal to experimental animals, histamine is essential for smooth regulation of key functions and thus survival. It could be compared to a thermostat in a heating system, which automatically corrects functional deficits to restore physiological balance.

L 2

Prostaglandin D_2: Mediator of inflammation and sleep as well as sensitive in-vivo marker of mast cell activation.

Sven-Erik Dahlén
The Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden.

Although most prostaglandins are ubiquities compounds being formed in many tissues in humans, prostaglandin D_2 (PGD_2) is in fact an exception by being almost exclusively formed at two sites. Quantitatively the by far dominating source is the mast cell but in the central nervous system prostaglandin D_2 is also formed in the specific pathways involved in sleep regulation. When mast cells are differentiated and activated there is a striking up regulation in the haemopoetic prostaglandin D synthase. Accordingly, following mast cell activation *in vitro* PGD_2 is released in association with histamine and newly formed mediators. Prostaglandin D_2 may act on several prostanoid receptors and in particular the TP receptor that mediates bronchoconstriction, the DP1 receptor that mediates vasodilatation and the CRTH2 receptor that mediates chemotaxis of several inflammatory and immune-competent cells. In addition, PGD_2 is also an agonist for certain EP receptors and the FP receptor. Recent evident from experimental models and human systems support that interference with prostaglandin D_2 actions has prominent effect on bronchoconstriction and airway inflammation. It has also been established that measurements of prostaglandin D metabolites such as 9α,11β-PGF_2 and 11-tetranor-PGDM represent an effective way to monitor mast cell activation *in vivo*. That measurement has been used to establish the involvement of mast cells in for example exercise-induced bronchoconstriction and aspirin intolerant reactions. In addition, measurements of PGD_2 and its metabolites are as of now the most sensitive method to determine mast cell activation for example in anaphylaxis and systemic mastocytosis.

L 3

Mast cells, nerves and IgE

John Bienenstock
Brain-Body Institute, St. Joseph's Healthcare Hamilton, Departments of Medicine and Pathology & Molecular Medicine, McMaster University
Hamilton, Ontario, Canada

The lecture will consider various aspects of mast cell and nerve interactions and the consequences of the cross-talk which is continually occurring between these two cell types. Mast cell-nerve association serves as a paradigm for neuroimmune communication and seems to be important both in terms of homeostasis and a variety of other cell and tissue systems. Most of the work which has been performed has concentrated on the consequences of these interactions both *in vivo*, *ex vivo* and *in vitro*. The conundrum of how and why mast cells appear to have specific associations with nerves in most tissues of the body, appears to be in part resolved through work showing that a molecule found in central nervous system synapses (SynCAM) may largely be responsible. However mast cells are also found in various parts of the central nervous system such as surrounding the pituitary gland. Here they can be responsible for antigen dependent regulation of HPA axis activity and also in response to stress. Recently high affinity IgE receptors have been demonstrated on pinealocytes where they may regulate some aspects of circadian rhythm activity and melatonin production. Furthermore we have shown the presence of IgE on neurites which can themselves be activated by antigen to which the IgE is directed. This raises an important biological question as to the evolutionary advantage of such tissue distribution and offers new interpretations and applications in neurogenic and allergic inflammation.

L 4

Differential release of mast cell mediators - implications in health and disease.

Gunnar Nilsson
Clinical Immunology and Allergy, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Mast cells are well known for their involvement in allergic reactions, where aggregation of IgE-receptors lead to degranulation (exocytosis) of the content of secretory granules together with release of lipid mediators (leukotrienes and prostaglandis) and secretion of *de novo* synthesized cytokines, chemokines and growth factors. To determine the degree of mast cell activation *in vitro* or *in vivo*, measurement of granule content (histamine and tryptase) or morphological assessment of degranulated mast cells are commonly used. Thus, mast cell degranulation is often taken as verification whether mast cells are activated or not. However, recent findings implicate mast cells also in diseases, such as multiple sclerosis and Hodgkin lymphoma, where mast cells appear to be intact by light microscopy. Today we know that mast cells can be activated to release inflammatory mediators without preceding degranulation. Several endogenous and exogenous stimuli have been described to cause release of lipid mediators and/or secretion of *de novo* synthesized mediators without release of e.g. histamine. Examples of endogenous stimuli are IL-1, SDF-1α, PGE_2 and CD30. The latter is a co-stimulatory molecule expressed on e.g. some sub-types of T-lymphocytes and the tumor cells in Hodgkin lymphoma. Treatment of mast cells with CD30 leads to a specific release of chemokines that are of importance for the recruitment of other inflammatory cells to the site of inflammation. CD30-treatment does not cause any release of histamine, tryptase or leukotrienes. Exogenous stimuli that cause degranulation-independent release of inflammatory mediators include products from pathogens that act via pattern-recognition molecules, where the Toll-like receptors are the most investigated. LPS and peptidoglycan acts on TLR-4 and TLR-2, respectively, leading to release of cytokines. In summary, the versatile roles of mast cells in different diseases and in host defense imply a tight regulation of the response to different stimuli. Differential release of mast cell mediators without degranulation has been stressed as critical in the pathogenesis of several inflammatory diseases, why it is important to decipher how mast cells can be activated and what they release.

L5

The role of serglycin proteoglycan in mast cell secretory granule formation

Gunnar Pejler
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Mast cells (MCs) are versatile cells of the immune systems, contributing in profound ways both to the innate and adaptive immunity. In addition, dysregulated actions of MCs can lead to harmful effects, as exemplified by anaphylaxis. When MCs are activated they may respond by degranulation, and in this process a number of preformed substances are released: histamine, serotonin, cytokines, serglycin proteoglycan and various types of proteases: chymases, tryptases and carboxypeptidase A (CPA). Serglycin proteoglycan consists of a small protein core to which densely sulfated and thereby negatively charged glycosaminoglycan (GAG) chains are attached, the GAG chains being of either heparin or chondroitin sulfate type. Serglycin is highly expressed in MCs but is also expressed by a multitude of other cell types, including neutrophils, platelets, macrophages and cytotoxic T cells. Targeting of the serglycin gene has pointed to a key role of this proteoglycan in the regulation of secretory granule maturation, both in MCs and other granulated hematopoietic cell types. Firstly, the absence of serglycin leads to completely abolished metachromatic staining of MC granules. Subsequent ultrastructural analysis revealed that, although granules are visible in serglycin-/- MCs, they lack the typical dense core formation seen in wild type MCs. Analysis of the different secretory granule compounds has shown that the levels of histamine and serotonin are profoundly decreased in serglycin-/- MCs, both in vivo and in bone marrow derived MCs. mRNA levels for the corresponding biosynthetic enzymes, HDC and TPH-1 are not affected, indicating an effect at the level of storage rather than synthesis. In analogy, the storage of several MC proteases including the tryptase mMCP-6, the chymases mMCP-4 and mMCP-5 and CPA is severely defective in serglycin-/- MCs. In contrast, the storage of other MC proteases (mMCP-1 and mMCP-7) is not affected by the lack of serglycin. Thus, serglycin has a key role in regulating the composition of mature MC granule, specifically promoting the storage of selected granule compounds whereas others are independent on serglycin for storage.

L 6

Intracellular trafficking and signaling mechanisms in the secretion of inflammatory mediators and histamine by mast cells.

Ulrich Blank
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Mast cells (MC) are professional secretory cells that contain histamine and other inflammatory products prestored in their cytoplasmic secretory granules (SGs). Stimulation through their high affinity IgE receptors (FcεRI) promotes massive degranulation and release of granular mediators such as histamine. This is followed by the secretion of multifunctional cytokines/chemokines. Although much information exists about early FcεRI-initiated signaling events, little is known about the late steps that govern membrane fusion of vesicular carriers. Previously, we and others have shown that degranulation involves SNARE membrane fusion proteins such as the plasma membrane localized target t-SNAREs SNAP-23 and syntaxin-4. Fusion accessory proteins such as syntaxin 3-interacting Munc18-2 were also shown to be implicated. However, the role of v-SNARE family proteins called VAMPs and localized on the vesicular carriers was less clear. Similarly, the mechanism of action of Munc18-2 is not well understood. In my contribution I will describe our data on the functional analysis of VAMP-8 and Munc18-2. Using VAMP-8-deficient bone marrow derived MCs (BMMCs), we observed that release of preformed mediators is inhibited compared to WT BMMCs. We found that VAMP-8-deficient mice have reduced plasma histamine levels in passive systemic anaphylaxis experiments, however, cytokine/chemokine release was not affected suggesting trafficking through different, possibly VAMP-3-containing vesicular carriers. Our findings demonstrate that VAMP-8 segregates secretory lysosomal SGs exocytosis in MCs from cytokine/chemokine molecular trafficking pathways. In addition, I will show that Munc 18-2 plays a positive role in degranulation and that its mechanism of action involves the interaction with cytoskeletal proteins.

L 7

Histamine dihydrochloride and interleukin-2 in acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is the most common form of acute leukaemia in adults. Although the majority of AML patients achieve complete remission (CR) after induction chemotherapy, a large fraction of patients will experience relapse of leukaemia, despite ensuing consolidation chemotherapy. The prospect of long-term survival after a relapse is poor, and there is a critical, and hitherto unmet, need for a safe and efficacious therapy to prevent relapse in AML.
The maintenance of remission in AML is dependent on the integrity of antileukemic lymphocytes such as natural killer (NK) cells and T cells. These effector lymphocytes have the capacity to attack and destroy residual leukaemic cells, and a conceivable strategy to maintain remission in AML is to enhance NK and T cell function by use of immunotherapy. Histamine has, unexpectedly, been found to efficiently protect human NK and T cells against functional inhibition and apoptosis inflicted by oxygen radical-producing myeloid cells. This protective property of histamine, which is strictly mediated by H2-type receptors on myeloid cells, has formed the basis for evaluation of histamine dihydrochloride (HDC) as a potential therapeutic agent in immunotherapy. In most studies, HDC has been combined with the prototypic NK and T cell activating cytokine interleukin-2 (IL-2). HDC/IL-2 results in a synergistic activation of NK and T cells *in vitro*, and the addition of HDC improves the immunostimulatory efficacy of IL-2 therapy *in vivo*.
In an international, randomised phase III trial (with 320 AML patients), treatment with HDC/IL-2 was found to prolong leukaemia-free survival (LFS; defined as the time from randomisation to relapse or death from any cause) over controls (no treatment; Log Rank Test p=0.008 for the ITT population of all patients randomised, p=0.01 for patient in their first CR [CR1]). At three years after randomisation, the percentage of relapse-free CR1 patients in the treatment arm was 40% as compared to 26% in control patients. The trial was well balanced for prognostic factors including age, prior therapy, leukaemic karyotype, FAB class, and time from CR to random assignment. The toxicity of HDC/IL-2 was acceptable: a) there was not treatment-related mortality, b) patients could safely administer HDC/IL-2 at home without medical supervision, c) ninety-two per cent of patients in CR completed all scheduled cycles of treatment, and d) no major treatment-related impact of quality-of-life was recorded. The benign nature of the treatment is corroborated by previous phase II data demonstrating that >70% of AML patients could return to gainful employment while receiving HDC/IL-2. These finding imply that HDC/IL-2 is an efficacious, yet safe and well tolerated remission maintenance therapy in AML.

Oral Communications

O1

Distinct signaling properties of human H_3 receptor isoforms.

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Histamine H_3 receptor (H_3R) ligands are beneficial in animal models of metabolic and cognitive disorders such as Alzheimer's disease and attention deficit hyperactivity disorder and have currently entered clinical trials. Since H_3R ligands may interact with multiple H_3R isoforms *in vivo*, we pharmacologically characterized several naturally occurring human isoforms, including the abundantly expressed splice variants $hH_3R(445)$ and $hH_3R(365)$, which differ by a deletion of 80 amino acids in the intracellular loop 3. We show that the $hH_3R(365)$ is differentially expressed compared to the $hH_3R(445)$ and has a higher affinity and potency for H_3R agonists and conversely a lower potency and affinity for H_3R inverse agonists. Furthermore, we show a higher constitutive signaling of the $hH_3R(365)$ compared to the $hH_3R(445)$ in both [^{35}S]GTPγS binding and cAMP assays, likely explaining the observed differences in hH_3R pharmacology of the two isoforms. Other lower expressing H_3R isoforms [$hH_3R(415)$, $hH_3R(413)$, $hH_3R(329)$] have also been identified that bind H_3R ligands and exhibit subtle differences in coupling to signaling mechanisms. These differences in H_3R pharmacology and signaling of the human H_3 receptor isoforms may be important for obtaining a detailed understanding of the physiological and potential therapeutic roles of H_3Rs.

O2

H_3 receptor agonists activate antiapoptotic pathways in rat cortical neurons and protect against neurotoxic insults.

Chiara Mariottini[1], [2]Tania Scartabelli, [2]Patrizio Blandina, [2]Domenico Pellegrini-Giampietro and [2]Maria Beatrice Passani.
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H_3 receptor (H_3R) ligands affect cognition, wakefulness, obesity conditions which are the main focus of research into the therapeutic potential of selective H_3R ligands. Here we demonstrate that H_3R activation protects cultured cortical neurons from neurotoxic insults such as starvation and excitotoxicity. Primary cortical neurons were prepared from ED17 Sprague-Dawley rats and maintained in culture for 6-8 days before being deprived of the nutrient B27 for 24h. Neurons were incubated for the whole period with the H_3R agonist immepip at concentrations ranging 1-100 nM. Cell viability was measured by MTT assay. Serum deprivation produced approx 30% cell death in 5 out of 9 cases, and this effect was dose-dependently attenuated by immepip. The same concentrations of immepip activated the Akt/GSK3β pathway in rat cortical neurons. To study excitotoxicity, cultures of mixed cells containing both neurons and glia were prepared from ED14 mice as previously described[1]. At day 14 in culture, cells were exposed for 10 min to NMDA (300 μM). Immepip was added to the medium 1h before NMDA and kept in the medium for the following 24h. The H_3R antagonist thioperamide (10 μM) was added to the medium 15min prior to immepip and then co-incubated with it. Cell damage was quantitatively evaluated by measuring the amount of LDH released from injured cells into the extra-cellular fluid 24 h following NMDA exposure. NMDA-induced neurotoxicity was significantly reduced by immepip (10,100 nM) inclusion in the incubation medium. This effect was blocked by thioperamide (n=4). The same concentrations of immepip activated the Akt pathway in mouse mixed cell cultures. The H_3R has been proposed as potential drug target for the treatment of various important CNS disorders including Alzheimer's disease and schizophrenia. Our results identify a new role for H_3R agonists as neuroprotective compounds.

[1]Pellegrini-Giampietro et al., 1999 Eur J Neurosci, 11:3637-3647

O3

The Effects of Ethanol on the Regulation of Motor Behaviour in HDC Knockout Mice.

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The role of histaminergic neurotransmission in alcohol-related behaviors is largely unknown. We have earlier shown that ethanol-insensitive rats display increased level of histamine in the brain and the reduction of brain histamine by alpha-fluoromethylhistamine treatment leads to an increased motor impairment induced by ethanol (Lintunen *et al.,* Neurobiol Dis, 2002). In the present study, we have further examined the role of brain histaminergic system in ethanol's effects on motor performance using male histidine decarboxylase (HDC) knockout mice. Firstly, the stimulatory effect of low-dose of ethanol was tested using locomotor activity measurement. Mice were acclimatized to the locomotor activity testing cages for two hours to remove the effect of a novel environment-induced exploratory behavior. Next, mice received an ethanol injection (1.5 g/kg, i.p.) and the locomotor activity was recorded for 20 minutes. Wild type animals showed a typical, short-lived increase in the locomotor activity at 5-15 min following ethanol administration. In contrast, HDC knockout mice differed significantly from the control animals showing hardly any stimulatory effect by ethanol. Secondly, the ability of mice to learn to stay for 180 s on an accelerating rotating rod (Rotarod) was tested in six trials per day on seven successive days. During the first day of training, young HDC knockout mice (3-6 months old) showed significantly superior performance on the Rotarod when compared to the wild type controls. This is in accordance with the study by Dere *et al.* (Eur J Neurosci, 2004). From the second day onwards, there was no difference in the motor behavior between the knockout and wild type animals. Interestingly, older HDC mice (15-17 months) did not show differences in their learning pattern between the genotypes. After the learning trials, the mice received ethanol injections in a cumulative manner (1-2.5 g/kg i.p.). No differences between HDC knockout and control mice were found in both young and old mice. The motor performance of HDC mice on a walking beam test was also studied both on young and old animals. We found no significant differences in the learning pattern or in the ethanol-impaired motor coordination between the genotypes in this test. These data suggest that histamine plays a role in ethanol-induced behaviors but in a complex manner. The ethanol-induced motor impairment in automated tasks seems to be histamine independent whereas ethanol's stimulatory effect on locomotion displays high dependence on brain histamine.

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O4

Immediate and delayed effects of metoprine injection in WAG/Rij rats.

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There is evidence that central histaminergic system is involved in the control of such brain functions as arousal, food and drink intake, exploratory behaviour, locomotion, and emotional state (Brown R. E. et al., 2001). It has been shown that metoprine (inhibitor of the histamine N-methyltransferase) significantly increased locomotor activity and the number of rearings in IRC mice (Sakai N. et al., 1992). The duration of clonic convulsions in the rats with electrically-induced seizures was significantly decreased (Yokoyama et al., 1992; Onodera et al., 1992) and the audiogenic convulsions were inhibited after metoprine injection (Tuomisto L. et al., 1987). The present study was aimed to assess the metoprine-induced changes of behaviour, EEG and food intake in WAG/Rij (absence epilepsy) and WAG/Rij-AS (mixed form of epilepsy, i.e. absence plus audiogenic epilepsy) rats. Administration of metoprine (10, 20 mg/kg) induced an extreme increase of running which was accompanied by automatisms and stereotyped behavior in a dose-dependent way in both rat groups. EEG records showed dose-dependent suppression of spike-wave discharges (SWD) which are characteristic for absence epilepsy and increase of summary duration of theta rhythm. WAG/Rij-AS rats lost body weight during the week after metoprine injection (20mg/kg). WAG/Rij rats on the contrary did not show weight loss. The results revealed that metoprine suppressed SWDs with concurrent increase of theta rhythm and active running. In addition metoprine injection caused decrease of food intake in WAG/Rij-AS rats.

O5

Citalopram, a selective serotonin reuptake inhibitor (SSRI), increases histamine release in the brain through its effect on endogenous 5-HT

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SSRIs are mainstay treatments for depression, and their highly specific actions enhancing 5-HT neurotransmission appears to explain their safety profile. Despite their claimed selectivity, however, SSRIs still interact, either directly or indirectly, with various neurotransmitter systems. The tuberomammillary nucleus (TMN) is the only source of histamine (HA) fibers, and receives serotonergic inputs from the raphé. To learn whether SSRIs affect the activity of HA neurons, citalopram was administered to rats and HA release was determined by microdialysis. SD male rats (250 g) were implanted with one probe in the TMN, and one in either the nucleus basalis magnocellularis or nucleus accumbens. HA output from the two probes, perfused with Ringer at 2-µl/min, was measured in 15-min samples by HPLC-fluorometric detection. Rats were conscious and moved freely in the cage. Spontaneous HA release from all regions was stable, ranging 0.05-0.08 pmol/15min (N=17). Intra-TMN administration of citalopram (1-10 µM) for 60 min increased significantly HA release up to about 100% of basal value from all areas ($P<0.05$, ANOVA/Fisher's test). Pretreatment with methysergide (10 µM), a 5-HT2 receptor antagonist, completely abolished the effect of 10 µM citalopram. These results suggest that citalopram activates HA neurons by increasing the extracellular levels of endogenous 5-HT, and provide evidence of functional connections between HA and 5-HT neuronal systems. Accordingly, earlier studies reported that local perfusion with 5-HT increased HA release from the anterior hypothalamus in anesthetized rats [1], and that 5-HT depolarized HA neurons [2]. Some effects elicited by SSRI (e.g.: sedation, tremor or weight gain/loss) may arise from the interaction of 5-HT with the histaminergic system.

[1] Laitinen et al, Eur J Pharmacol 1995; 285:159-64.
[2] Erikson et al, Neuropharmacology 2001; 40:345-51

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O6

Further Evidence for H_4 Histamine Receptor Expression on Central Neurons.

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The histamine H_3 receptor (H_3R) is a presynaptic auto- and hetero-receptor, reported to be abundantly expressed in the central nervous system of different mammalian species, including human, while the presence of the histamine H_4 receptor (H_4R) on neurons remains controversial. We have previously reported (Chazot et al., EHRS 2006) the first evidence for the expression of histamine H_4R in the human central and rat enteric nervous system, using slice immunohistochemical (IHC) and immunoblotting (IB) methods with our anti-hH_4R (374-390) antibody.
Based on IHC, we show that the H_4R displays a unique expression profile in the murine brain, including the deep laminae of the cerebral cortex. Follow-up slice electrophysiological studies demonstrated for the first time that the H_4R is *functionally* active on cortical neurons in the mouse. Recently, primary E17 rat cortical neuronal cultures were prepared and probed for H_3 and H_4 receptor expression using IHC and IB at DIV 14 and DIV 21, with our anti-H_3R and H_4R antibodies raised in rabbits, affinity purified and validated as previously described (Cannon, Chazot et al., 2006; van Rijn, Chazot et al., 2006). Both receptor types were detected in the cultures, the H_3R being more prevalent than the H_4R, consistent with previous mRNA studies. Based on quantitative IB, a major Mr 75,000 immunoreactive (H_4-IR) species was detected at both time points in culture, co-incident with the native mouse and rat brain membrane species and recombinant *dimeric* human H_4R expressed in HEK 293 cells. Interestingly the expression of this H_4-IR species at DIV 21 was significantly higher than at DIV14, once standardized for protein levels with ß-actin.
Herein, we provide the first evidence that the H_4R is functionally expressed on neurons in native brain slices. Furthermore, this study also suggests that the H_4R protein is differentially expressed in the mouse brain and developmentally regulated in rat neuronal cortical cultures. This provides new tools to study the physiological relevance of the H_4 receptor in the brain.

KE Cannon, PL Chazot, V Hann, FC Shenton, LB Hough, FL Rice (2006) *Pain* **129**, 76-92.
PL Chazot, FC Shenton, H Waldvogel, D Grandi and G Morini (2007) *EHRS conference* Florence, Italy.
R van Rijn*, PL Chazot*, FC Shenton, RA Bakker and R Leurs (2006) *Mol. Pharmacol.* **70**, 604-615.

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O7

PF-2988403 – an 'H_4 antagonist' demonstrating the full range of in vitro pharmacologies which translate *in vivo* in the rat.

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BACKGROUND. Expression of the histamine H_4 receptor is mainly restricted to bone marrow, spleen and circulating leukocytes, and thus represents an anti-inflammatory target. The distribution of the target is broadly conserved across species; however the sequence homology is not. Hence, diverse ranges of potency and functional effects across the species by the same compound could be possible.
OBJECTIVE. PF-2988403 is a novel selective H_4 antagonist and here we describe its' *in vitro* and corresponding *in vivo* profile in the rat.
METHODS. *In vitro* potency and selectivity was assessed in HEK-293T cells expressing either the human recombinant $H_{1/2/3/4}$ receptors separately or the mouse, rat, guinea-pig, dog or monkey H_4 receptor using inhibition of [^{3}H]-pyrilamine (H_1), tiotidine (H_2), N-a-methyl histamine (H_3) and histamine (H_4) binding. The functional effects were assessed by stimulating the cells with forskolin and tested as agonist/inverse agonist and as antagonists of a single concentration (~ED_{80}) of histamine. *In vivo*, the oral administration of PF-2988403 at doses from 2.6 to 720 mg/kg/day for 4 days to male rats was explored to determine any physiological or pathophysiological effects.
RESULTS. PF-2988403 produced concentration related changes in [^{3}H]-ligand binding to all human histamine receptor subtypes with Ki's of 9.55 nM (5.19-17.6; n=5) at H_4, and >2uM at H_1, H_2 and H_3. The profile of PF-2988403 differed at human and animal H_4 receptors, exhibiting the full range of functional effects, from inverse agonist (human H4), to neutral antagonist, to partial agonist and full agonist (rat H_4). *In vivo* in the rat, the effects were consistent with a full agonist, i.e. pro-inflammatory effects (e.g. changes in peripheral blood / bone marrow and spleen), thereby supporting the theory that an H_4 antagonist would be anti-inflammatory.
CONCLUSIONS. PF-2988403 is an 'H_4 antagonist' demonstrating the full range of *in vitro* pharmacologies which translate *in vivo* in the rat.

O8

Could Antidepressants Noticeably Affect Histamine Degrading Enzymes and Consequentially Inflammatory Processes? A Study in Guinea Pig and Rat.

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In the mammalian body, histamine is effectively metabolised by two enzymes: diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT). It has been shown that DAO has a protective function in small intestine and placenta, and possibly therapeutic role in allergy in guinea pigs. HNMT is important histamine degrading enzyme in brain and lung, moreover, it has been shown that patients with asthma have low HNMT activity in the lung. Our previous studies with antidepressants showed that they affect histamine pharmacokinetics. Therefore we examined whether antidepressants affect histamine degrading enzymes what could also contribute to inflammation reduction. First we measured basal DAO and HNMT activity and their mRNA expression in different guinea pig and rat tissues to supplement incomplete data from the literature. Next, guinea pigs were treated with 1 or 2 doses of amitriptyline (AMI) and we followed the release of DAO into plasma, changes in enzyme activity and mRNA expression. In the *in vitro* study with different concentrations of AMI and sertraline (SER) we established the effect of antidepressants on rat and guinea pig DAO and HNMT activity at the molecular level. The results revealed that 1 or 2 doses of AMI *in vivo* significantly lowered the rate of heparin-induced release of DAO. We proved that AMI diminished DAO release from small intestine, liver and lung. AMI also augmented mRNA expression, as well as DAO and HNMT activity in guinea pig tissues. Both antidepressants altered DAO and HNMT activity at the molecular level, the effect was concentration- and animal species-dependent. The *in vitro* results suggest a minor overall influence of antidepressants on histamine degrading enzymes in the rat and more prominent effect in the guinea pig. Our multipart study clearly demonstrates that antidepressants, particularly AMI enhance the histamine degrading processes in guinea pig, what could beneficially contribute in allergy treatment.

O9

Probing the histamine H_4 receptor binding site: from fragments to active ligands.

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The human histamine H_4 Receptor (hH_4R) belongs to the family of G protein-coupled receptors (GPCRs), an attractive and proven class of drug targets in a wide range of therapeutic areas. Using a fragment based approach and a pharmacophore model we have designed, synthesised and evaluated a series of compounds at the hH_4R from which 2-(4-methyl-1-piperazinyl)-quinoxaline was identified as a new lead structure for H_4R ligands. Exploration of the structure-activity relationship (SAR) of this scaffold led to the identification of potent H_4R ligands with nanomolar affinities. These compounds were evaluated for their affinity against the other histamine receptor subtypes and were found to behave as potent and selective H_4R ligands. *In vivo* studies in the rat reveal that some of these ligands show significant anti-inflammatory properties in the carrageenan-induced paw-edema model.

O10

A selective H_4R antagonist prevents antigen-induced asthma-like reaction and airway inflammation in guinea pigs.

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Background: The histamine H_4 Receptor (H_4R) is a novel G-protein coupled receptor expressed on dentritic cells and on inflammatory cells involved in asthma. Although mechanisms involved in the pathogenesis of asthma remain unclear, roles for oxidative/nitrative stress, epithelial cell apoptosis and airway inflammation have been documented. Ceramide is a second messenger molecule modulating cell apoptosis and oxidative stress, and ceramide *upregulation* could contribute to airway hyperreactivity and inflammation during asthma. In this study, we evaluated the effects of compound JNJ 7777120, a selective H_4.receptor antagonist, on antigen-induced asthma-like reaction in sensitized guinea pigs, and we tested the ability of fumonisin B1, a competitive and reversible inhibitor of ceramide synthase, to modulate the effects of compound JNJ7777120.
Methods: Ovalbumin-sensitized guinea pigs placed in a respiratory chamber were challenged with the antigen. JNJ7777120, 5 mg/kg b.wt., was given i.p. for 4 days before ovalbumin challenge. Sixty min before JNJ7777120 administration, some animals were treated i.p. with FB1 (1 mg/kg b. wt.). Respiratory parameters were recorded and quantified. Lung tissue specimens were taken for histopathological and morphometric analyses and for ceramide and eosinophilic major basic protein immunohistochemistry. Moreover, myeloperoxidase activity, 8-hydroxy-2-deoxyguanosine, MnSOD, were evaluated in lung tissue extracts. In the BAL fluid, the levels of PgD_2 and TNFα were measured.
Results: Ovalbumin challenge increased ceramide levels and ceramide synthase activity in the airway epithelium, associated with respiratory abnormalities, such as cough, dyspnea and severe bronchoconstriction. Treatment with JNJ7777120 significantly reduced these abnormalities. Pretreatment with FB1 increases the protective effects of compound JNJ7777120.
Conclusion: The results here reported clearly show that H_4R antagonism modulate allergic asthmatic response and airway inflammation, these effects are potentiated by manipulating ceramide formation.

O11

Impact of H 3 /H 4 antagonists on IgE and IgE-regulatory cytokine synthesis.

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Withdrawn

O12

Microarray profiling and pathway analysis of colorectal cancer.

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Production of histamine in colon tumours has been described earlier. Histamine-mediated signals have been shown to be implicated in tumour growth, and the effects of histamine is largely determined by the histamine receptor expression pattern locally. The authors analyzed histamine receptor expression in human colorectal cancer, adenoma and normal mucosa by quantitative reverse transcription-polymerase chain reaction, Western blot analysis and immunostaining. Real-time RT-PCR results revealed significantly decreased ($p<0.001$) H_1R and H_4R mRNA levels compared to normal colonic mucosa, without any significant change in H_2R mRNA expression. H_3R was absent in most samples, it was detected at low levels. Protein analysis showed a similar decrease of histamine receptor expression in carcinoma compared to normal mucosa controls. Immunohistochemical staining revealed expression patterns of H_1R, H_2R and H_4R similar to those suggested by the mRNA and Western blot results. In the present study, we demonstrated that H_1R, H_2R and H_4R are expressed in colon carcinoma and the adjacent normal mucosa. Based on these results, we performed further microarray analysis on Dukes' classified and selected tumours and adjacent normal mucosa samples. We carried out two colour-microarray experiment comparing Dukes' B (lymph node negative) and Dukes' D (distant metastasis positive) tumor and adjacent normal mucosa samples. The results were analyzed by Ingenuity Pathway Analysis software and we found a canonical pathway that the decreased level IGF-1 in tumour tissue correlated with the lower level of MDR-1 both in mRNA and protein levels. We provide evidence that recombinant human IGF-1 increase the expression and activity of MDR-1 in human colon cancer cell lines via the MAPK cascade.

O13

Mast cell degranulation and mediator secretion in response to hypoxia - implications in host defence.

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Background: In response to different inflammatory conditions such as atherosclerosis inflammation, infections and in rheumatic joints, oxygen level drops and tissue becomes hypoxic. Hypoxia may affect the secretion of several cytokines, chemokines and angiogenetic factors due to stabilisation of the hypoxia inducible factor (HIF-1α) which regulates the expression of several hypoxia responsive genes. The effects of low oxygen levels on mast cells are not well established.
Aim: To investigate if mast cell secretion and degranulation is affected by hypoxia.
Methods: Cord blood derived mast cells (CBMC) was subjected to three different culturing conditions; 1) Cells cultured and stimulated in normoxia (21% O_2). 2) Cells cultured in normoxia and stimulated in hypoxia (1% O_2). 3) Cells cultured in hypoxia and stimulated in normoxia. Mast cells were stimulated with secretagogues acting via different pathways such as anti-IgE, calcium ionophore A23187, lipopolysaccaride (LPS) and CD30/Fc chimera. The amount of degranulation and mediator release was analysed by ImmunoCap, ELISA and CBA.
Results: Hypoxia *per se* does not induce mast cell degranulation or secretion of cytokines (IL-8 or IL-1β). However, mast cell reactivity was affected by hypoxia with decreased release of IL-8, whereas degranulation and release of IL-1β was less affected by hypoxia compared to normoxia. Thus, our data suggest that CBMC degranulation and cytokine release are sustained by hypoxia in response to different stimuli.
Conclusion: Our results imply that mast cells may have important impact in host response since they are stable to environmental changes and at the same time able to be reactivated by external triggering factors.

O14

Adenosine: Role of Different Receptor Subtypes in Mediating Histamine Release from Human and Rodent Mast Cells.

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Adenosine, an endogenous nucleoside, exerts a modulating effect in a large number of cellular systems by acting through specific receptors (adenosine A_1, A_{2A}, A_{2B} and A_3 receptors) that belong to the G-protein-coupled receptor family. Adenosine provoked bronchoconstriction in asthmatic patients with mast cells as an intermediate. Mast cell activation mediated by FcεRI is a critical event in the allergic inflammatory response and the actions of adenosine on human culture mast cell (HCMC) and rat peritoneal mast cell (RPMC) activation were investigated and compared in the current study. Histamine release assay was employed as the major indicator for mast cells activities and histamine content were measured spectrofluorometrically by a Bran+Luebbe AutoAnalyzer 3. Adenosine and analogues alone were inadequate to induce HCMC and RPMC degranulation. However, adenosine when added to immunological activated mast cells, modulation of histamine release was observed. In HCMC, a biphasic response with potentiation at low dose (10^{-8} M) and inhibition at high dose (10^{-5} – 10^{-4} M) was obtained, while in RPMC, opposite actions were demonstrated. Studies on adenosine agonists as well as antagonists revealed that the potentiation by adenosine in HCMC was mediated by the A_1 receptor and inhibition was mediated through the A_{2B} receptor. In contrast, inhibition on anti-IgE-induced histamine release at a low dose of adenosine was mediated by the A_{2A} receptor in RPMC, potentiation at a high dose was a action of the A_3 receptor. The current study revealed that heterogeneity of mast cells demonstrated different characteristics of adenosine receptor biology. The involvement of distinct receptors indicates a complex network of receptor interactions. Dual action of adenosine on mast cells provides flexibility in rational exploitation of these receptors as therapeutic targets on mast cells related to allergic and inflammatory disease.

O15

Effects of varying pH on IgE-dependent histamine secretion from human basophils.

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Previous reports have shown a decrease in pH within the respiratory tract of chronic asthmatic patients. In the current study, we investigated the effect of low pH on IgE-dependent mediator secretion from human basophils, cells which are thought to play a major immunomodulatory role in chronic allergic inflammation. Human basophils were enriched by means of Ficoll density centrifugation and, in some experiments, further purified to homogeneity by negative selection using a commercially available magnetic cell sorting kit. Basophils were treated with HEPES-buffered Tyrode's solution of varying pH (4.6 - 7.4) and preincubated for 15 min before stimulation with anti-IgE. Histamine release was assessed spectrofluorometrically. Low pH buffer gave rise to diminished anti-IgE induced histamine releases (1.2 ± 1.0 % mean net release at pH 4.6 compared to 18.1 ± 2.7 % at pH 7.4). Similar data were obtained with other secretagogues (concanavalin A and fMLP) and the inhibitory effects of low pH buffer were found to be reversible after resuspending the cells in buffer of normal physiological pH before stimulation. Viability, assessed by Trypan Blue exclusion, was not greatly affected by incubating the cells for up to 1 hour in low pH buffer. The mechanisms responsible for reduced basophil mediator release at low pH have not yet been elucidated. However, our data strongly suggest that reduced pH within chronically inflamed tissues may serve as an important control to regulate the pro-inflammatory and immunomodulatory actions of allergic effector cells such as human basophils.

O16

Histamine intolerance: overestimated or underestimated?

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Histamine is an important mediator of a wide variety of physiological reactions but it can also cause undesirable effects if produced in excess or if not inactivated properly. The term histamine intolerance has been coined to explain various symptoms that appear to be caused by dietary histamine. Generally, histamine intolerance describes a state where the catabolic capacity for endogenously released or exogenously administered histamine is insufficient leading to histamine mediated adverse reactions.

Histamine can be inactivated by two pathways: methylation of the imidazole ring by histamine N-methyltransferase (HMT) or oxidative deamination of the primary amino group by diamine oxidase (DAO). Whereas HMT as a cytosolic enzyme is responsible for the inactivation of intracellular histamine, DAO as a secretory protein will act mainly on extracellular histamine. Since the expression levels and tissue activities of both enzymes show considerable individual variation in humans it is conceivable that some people may indeed have an insufficient histamine degradation capacity in case of strong endogenous release or significant exogenous uptake.

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. Therefore, diagnosis of histamine intolerance must exclude other causes and should include oral histamine provocation with determination of resorption and associated symptoms. Until the effectiveness of oral DAO supplementation introduced recently has been demonstrated, the main therapy remains avoidance of histamine containing food.

O17

Effects of histone deacetylase inhibitors on rat mast cell activation and rat colonic smooth muscle.

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Histone deacetylase inhibitors (HDACis), novel anticancer drugs, may have anti-inflammatory effects. They seem useful in inflammatory bowel disease (IBD)[1] and inhibit dextran sulfate-induced colitis in the rat and mouse, animal models of IBD.The mechanism of this inhibition is unknown, and so is the effect of HDACis on colon contraction. We tested a selection of HDACi for their effect on rat colon smooth muscle contraction and mast cell histamine release (HR): suberoylanilide hydroxamic acid (SAHA), its diamide analogue, diamide (1), MGCD0103 and sodium butyrate (short-chain fatty acid derivative, CFA).
These HDACi had no effect on colon contraction by theophylline (non-selective purinergic P_1 antagonist), AAPH (peroxyl radical generator) or carbachol, while NaF-induced contraction was blocked by SAHA (maximal inhibition 50 % at 1 μM), MGCD0103 (maximal, *ca.* 50 % at 0.1 μM) and sodium butyrate (1 mM) but not diamide (1); whereas histone-induced contraction was only reduced by SAHA. Butyrate itself at and above 1 mM caused some contraction. Both the NaF- and histone-induced contraction was blocked by benzalkonium chloride, while inhibition NaF-induced contraction by HDACis was blocked by NO synthase inhibitors and propranolol. RPMC histamine release (HR) by antigen (RPMC passively-sensitized with mouse monoclonal antigen-specific IgE antibody) or NaF (G-protein activator), but not compound 48/80, or exogenous histone [2] was inhibited by MGCD0103. The effect of SAHA, diamide (1) or butyrate on HR was insignificant.The above effects of brief preincubation (15–30 min) with HDACis may be through interference with G-protein-coupled-receptor mechanisms (including SCFA orphan GPCR 43 or GPCR41 on mucosal enterocytes, enteroendocrine cells and mast cells, which regulate contraction via neural reflexes [3]), rather than gene expression, though induction of NO synthase may be involved in HDACis inhibition of NaF-induced contraction.

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O18

Toll-like receptors and RBL-2H3 mast cells.

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Mast cells are involved both in the development of allergy and in the initiation of the innate immune response. Mast cells can be activated during immune responses by several mechanisms, including the linkage of different pathogen-associated proteins to certain pattern recognition receptors called Toll-like receptors (TLRs). In mast cells, TLR2-mediated activation leads to degranulation while TLR4-mediated activation leads to the production of newly synthesised cytokines such as TNF-α and IL-13. It is well known that lipopolysaccharide (LPS) can activate mast cells through their TLR4 receptors. As part of an ongoing study to assess the anti-inflammatory properties of novel chemical entities, LPS was used to activate the rat basophilic leukaemia mast cell line, RBL-2H3. Responsiveness of RBL-2H3 cells to LPS was assessed by TNF-α and IL-13 production using commercially available ELISA kits. LPS at concentrations between 0.1-1 μg/ml failed to produce a response from RBL-2H3 cells. The amount of IL-13 released after stimulation with 1 μg/ml of LPS was 35±29 pg/ml which was not significantly ($P<0.05$) different from the control (39±24.86 pg/ml), while the released TNF-α was 0±13 pg/ml. To verify the expression pattern of TLRs in RBL-2H3 cells, the presence of mRNA transcripts was assessed by using RT-PCR. Transcripts encoding TLR3, 4, 5 and 6 was detected in RBL-2H3, while messages for TLR1, 2, 8, 9 and 10 were not found.
RBL-2H3 cells were unresponsive to LPS although TLR4 mRNA was present in the cells. Our results are inconsistent with previously published data where others have shown RBL-2H3 releasing up to 180 pg/ml of IL-13 and 60 ng/ml of TNF-α [1]. However, being generated from a solid basophil-like tumor, RBL-2H3 cells share many similarities with basophils and this resemblance might explain the lack of response; it has been shown that basophils in primary culture express TLR4-receptors but do not exhibit a response after stimulation with LPS [2]. Moreover, the lack of TLR2 in RBL-2H3 cells suggests a possible unresponsiveness to bacterial components, such as PGN, challenging this cell line as an *in vitro* model to study mast cell biology.

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O19

IL-18 stimulates Ccl1 expression in mouse non-activated mucosal mast cells

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In our previous microarray studies the IL-18Ra chain was among the most upregulated genes during mouse mucosal mast cell (MMC) *in vitro* differentiation. Activated macrophages, dendritic cells, airway epithelial cells and keratinocytes are the primary sources for IL-18 that induces IFN-γ production from Th1 and NK cells in the presence of IL-12. However, in the absence of IL-12, IL-18 results in the expression of Th2-type cytokines and IgE. Although IL-18 has been proven to induce IgE-independent IL-13 production in mast cells and basophil granulocytes in the presence of IL-3, the effect of this cytokine on mast cells is not well understood.
In our experiments we studied the IL-18-induced gene expression changes in *in vitro* differentiated MMCs. Expression microarray data analysis resulted in >100 differentially expressed genes after a short stimulation period. The evaluation of this gene group by Ingenuity Pathway analysis resulted in the identification of a chemokine group (Ccl1, -3 and -5) belonging to the same network. We proved that IL-18 enhances Ccl1 production transiently and dose-dependently through the NFkappaB signalling pathway not only in MMCs, but also in immature cells. Interestingly, the Ccl1-inducing effect of IL-18 was not detected in connective tissue-type mast cells. Nitric oxide, that is known to prevent the activation of NFkappaB and is an important mediator produced by airway and lung epithelial cells in asthmatic individuals, inhibited the IL-18 induced Ccl1 expression in MMCs. Furthermore, IL-18 did not have a great influence on the gene expression profile in IgE/antigen activated MMCs.
Our results show that IL-18 induces IgE/antigen-independent Ccl1 production in MMCs, but not in peritoneal mast cells. As IL-18 is expressed by stimulated airway epithelial cells, this raises the possibility that the IL-18 induced Ccl1 production of MMCs may contribute to the onset of mucosal inflammation by attracting Th2 cells through their surface Ccl1 receptor CCR8.

O20

Residue 6.45 in the histamine H_1 receptor acts as a switch for ligand-mediated activation.

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The human histamine H_1 Receptor (hH_1R) belongs to the family of G protein-coupled receptors (GPCRs), an attractive and proven class of drug targets in a wide range of therapeutic areas. Using random mutagenesis methodology, we previously identified several highly Constitutively Active Mutants (CAMs) of hH_1R upon mutation at position 6.40 and 6.45 in transmembrane VI [Bakker et al, 2008, *Mol Pharmacol.* **73**]. In this study we found that mutation of residue 6.45 does not only result in a CAM ($Ile^{6.45}Asn$) but also a mutant that does not respond to the endogenous ligand histamine ($Ile^{6.45}Arg$) in CRE-Luc and NFκB reporter gene assays. Surprisingly, this $Ile^{6.45}Arg$ mutant could be activated by only a particular class of synthetic H_1R inverse agonists that contain tricyclic moieties such as doxepin and ketotifen. These data suggest that the tricyclic compounds act differently from the biaryl class of H_1 inverse agonists. These pharmacological data, in combination with molecular modeling study will provide better insight on hH_1R ligand binding sites and receptor activation mechanism.

O21

Altered T Cell Nitric Oxide Production and Cytokine Production in Histidine Decarboxylase Knockout Mice.

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Histamine is a key regulator of the immune system. Several lines of evidence suggest the role of histamine in T cell activation and accelerated Th1 immune response is a hallmark of histidine decarboxylase knockout (HDC-KO) mice with complete lack of endogenously produced histamine. According to our previous work, T lymphocytes produce nitric oxide (NO) upon activation and NO is necessary for effective T cell activation. In order to study the role of histamine in T cell activation, we investigated cytokine production and T cell signal transduction in HDC-KO and wild type mice. In the absence of histamine, an elevated INF-γ mRNA and protein levels of splenocytes ($p<0.001$; $p=0.001$ respectively) were associated with a markedly increased (2.5-fold, $p=0.0009$) NO production, compared to wild type animals. Furthermore, histamine treatment decreased the NO production of splenocytes from both wild type and HDC-KO mice ($p=0.001$; $p=0.0004$, respectively). NO precursor (Z)-1- [2-(2-aminoethyl) - N - (2-ammonioethyl) amino] diazen - 1-ium-1,2- diolate- diethylenetriamine (NOC-18) elicited IFN-γ production ($p=0.0002$), while NO synthase inhibitors, NG-monomethyl-L-arginine and nitronidazole both inhibited IFN-γ production ($p=0.002$ and $p=0.01$ respectively), suggesting the role of NO in regulating IFN-γ synthesis. Cytoplasmic Ca2+ concentration of unstimulated T cells was increased in the HDC-KO mice ($p=0.02$), while T cell activation-induced delta Ca2+-signal was similar in both HDC-KO and wild type animals. Our present data indicate that, in addition to its direct effects on T lymphocyte function, histamine regulates cytokine production and T cell signal transduction through regulating NO production.

O22

Polymorphisms in Murine Histamine Receptor H_1 Lead to Differential Cell Surface Expression and Influence Autoimmune Disease Progression.

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Structural polymorphisms (L263P, M313V and S331P) in the third intracellular loop of the murine histamine receptor H_1 (H_1R) are candidates for Bphs, a shared autoimmune disease locus in experimental allergic encephalomyelitis (EAE) and experimental allergic orchitis. The P-V-P haplotype is associated with increased disease susceptibility (H_1RS), whereas the L-M-S haplotype is associated with less severe disease (H_1RR). Here, we show that selective reexpression of the H_1RS allele in T cells fully complements EAE susceptibility and the production of disease associated cytokines while selective reexpression of the H_1RR allele does not. Mechanistically, we show that the two H_1R alleles exhibit differential cell surface expression and altered intracellular trafficking, with the H_1RR allele being retained within the endoplasmic reticulum (ER). Moreover, we show that all three residues (L-M-S) comprising the H_1RR haplotype are required for altered expression. These data are the first to demonstrate that structural polymorphisms influencing cell surface expression of a G-protein coupled receptor in T cells regulates immune functions and autoimmune disease susceptibility.

O23

Differential Voltage-Sensitivity of Human Histamine H_3 and H_4 Receptors.

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Background
Recently, ligand binding affinity and G-protein coupling of some neurotransmitter receptors have been shown to be regulated by voltage, a mechanism which has been suggested to play a crucial role in the regulation of neurotransmitter release by autoreceptors. We have investigated whether the histamine H_3 receptor, which functions as an inhibitory auto- and heteroreceptor in nerve terminals of the central nervous system, is sensitive to the membrane potential. We have also assessed the corresponding sensitivity of the closely related histamine H_4 receptor, which is predominantly expressed in hematopoietic cells.
Objectives
To evaluate the putative effect of membrane depolarization on the concentration-response relationships of histamine H_3 and H_4 receptor-induced activation of G protein-coupled inward rectifier potassium channels (GIRK).
Methods
Xenopus oocytes were injected with cRNA encoding histamine H_3 or H_4 receptors along with cRNA encoding GIRK channel subunits. Electrophysiological recordings were performed using the two-electrode voltage-clamp technique. Briefly, oocytes were superfused with high potassium (25 mM) solution and GIRK currents evoked either by a hyperpolarizing step to -80 mV, or by a depolarizing step to +40 mV. The hyper- or depolarizing potential was maintained throughout application and washout of histamine receptor ligands.
Results
Comparison of concentration-response relationships at -80 mV and at +40 mV for histamine H_3 receptor-induced GIRK activation revealed rightward shifts of near tenfold for both histamine and R-alpha-methylhistamine upon depolarization. In contrast, the concentration-response relationships for H_4-induced GIRK activation were not affected by transmembrane voltage to any appreciable extent.

O24

Subcellular Localization of Histamine H3 Receptor in Human Mammary Cells.

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We have reported the expression of histamine (HA) H_3 and H_4 receptors (H_3R, H_4R) in non-tumorigenic (HBL-100) and tumorigenic (MDA-MB-231) breast cell lines and also in benign and malignant lesions from human mammary gland. In MDA-MB-231 cells, HA modulates proliferation in a concentration-dependent manner through H_3R and H_4R. In this work, we evaluated the modulation of the H_3R expression and its cellular localization after treatment with HA, H_3R ligands or starvation in MDA-MB-231 cells. The cellular localization of H_3R was determined by immunocytochemistry using two antibodies (Alpha Diagnostic and Sigma) against the human H_3R and FITC-conjugated secondary antibody. On intact and permeabilized cells, nuclei were stained with ethidium bromide and fluorescence was observed under confocal microscopy. The semiquantification was performed by flow cytometry. Results demonstrate a very low H_3R detection in intact cells while, permeabilization allows its detection. Only a few untreated permeabilized cells showed H_3R immunoreactivity especially with cytoplasmic localization. Serum deprivation and 10 μM HA treatment considerable increased the H_3R expression in all cells, within the intracellular compartment with nuclear and perinuclear localization. 10 μM Imetit and Clobenpropit addition increased the H_3R expression. The former with H_3R immunoreactivity localized in nucleus and preferentially in plasma membrane while the later augmented the H_3R expression with nuclear and perinuclear localization. This intracellular localization of the H_3R was confirmed in permeabilized HBL-100 cells and in benign and malignant breast lesions determined by immunohistochemistry. To our knowledge, this is the first report that describes the modulation of the H_3R expression and cellular localization of the human H_3R under different proliferative stimuli. The precise mechanism involved in these effects remains unknown and may represent a novel way for the regulation of H_3R signaling.

O25

Systemic Challenge with Lipopolysaccharide Induces Histamine Levels in the Rat Conjunctiva and Cartilage but not in the Hypothalamus.

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Histamine (HIS) is a key mediator stored and released upon mast cell (MC) activation in virtually every organ including the eye, while additional sources comprise central neurons and cartilage. As opposed to the mechanism employed by MCs, the characteristics of HIS supplied by other cells are the rapid formation and release and the association with inflammation exacerbation. Lipopolysaccharides (LPS) are proinflammatory bacterial components that stimulate the mobilisation of diverse mediators including HIS, while as potent histidine decarboxylase inducers LPS may play a role in HIS formation. This study sought to investigate the effects of LPS on the HIS content of the rat conjunctiva, cartilage and hypothalamus. Adult male Sprague Dawley rats were divided into 2 groups (n=5 each), one receiving 100μg/kg b.w. i.p. LPS (*E. coli* serotype 026:B6) in sterile, pyrogen-free 0.9% saline and the control group saline alone. Following decapitation after 2h, an end-point coinciding with peak central and peripheral responses, the conjunctivae, cartilaginous parts of the 9th-10th costosternal junction and the hypothalami were removed; HIS was extracted and quantified fluorometrically. The conjunctival and cartilage HIS levels were significantly lower (p<0.05) than the respective values in Wistar rats, but hypothalamic HIS (HHIS) levels were similar (p>0.05) in these strains. In LPS-treated rats conjunctival and cartilage HIS content was significantly increased (p<0.05) to 204±8 and 186±19% of the control, respectively, while HHIS levels were comparable to control (p>0.05). In conclusion, systemic inflammatory challenge with LPS induced HIS levels in peripheral tissues but not in the hypothalamus. Considering the reported decrease in tissue HIS levels upon C48/80 challenge, the data are indicative of a MC degranulation-independent process. The elucidation of potentially selective underlying mechanisms in peripheral and brain function during infection and systemic inflammation are in progress.

O26

Th17 in sensitized human lymphocytes ex vivo.

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Human sensitized T-lymphocytes reveal increased STAT6/STAT1-balance corresponding to DNA-promoter gene interaction compared to normal controls. As IL-17E has currently been identified as mediator of tissue inflammation, the aim of this paper was to investigate the presence of this factor in circulating human lymphocytes *ex vivo*. Human PBMC from a group of 12 atopic/non-atopic volunteers (IgE > 500 IU, normal age matched controls: < 50 IU) were stimulated by anti-CD3 in 3-day cultures. Allergic patients were suffering from seasonal hay fever and chronic atopic eczema. Histamine was added 4 hours post-plated. Cytokines were measured by the ELISA-, STATs by Western-blot- and STAT-DNA-interaction by the electrophoretic mobility shift assay (EMSA)-technique. For EMSA the oligonucleotides STAT3 5´GAT CCT TCT GGG AAT TCC TAG ATC 3´ (LI-COR USA) were applied. PBMC differentiation was identified using cytoflowmetric/FACS-analyses. STAT6 as well as phosphorylated (activated) aSTAT6 were stimulated after 1-day culture time in sensitized cells with increased CCR3-expression inducing IL-4-expression and -secretion. Western blots showed delayed STAT3 increase. No significant differences could be observed between the atopic and the non-atopic group. ($p > 0.05$, $n = 6$) The STAT3 concentrations were in a good correlation to IL-17E. In the presence of histamine, STAT3 was suppressed. In contrast, IL-17E did not respond to different histamine concentrations added to anti-CD3-stimulated cell cultures. However, histamine could specifically influence STAT3-interaction with sensitized lymphocyte promoter gene as it was proved using EMSA. It could be concluded that Th17 showed downstream regulatory effects which were specific for atopic pathology. Whether IL-17E is a key cytokine in allergy disease must be elucidated in future work.

O27

Effects of a selection of histone deacetylase inhibitors on antigen- and agonist-induced airway smooth muscle contraction

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Histone deacetylase inhibitors (HDACis), novel anticancer drugs, are thought to have anti-inflammatory effects, as suggested by studies in animal models of autoimmune diseases, asthma and inflammatory bowel disease (IBD), and by clinical trials in IBD [1]. Accordingly, *in vitro* studies related to two of these models, one of asthma and another of IBD, were conducted on a selection of HDACis: the two hydroxamic acids, suberoylanilide hydroxamic acid (SAHA, Vorinostat [TM], 'Zolinza'[TM], now undergoing clinical evaluation in cancer), and a newly synthesized 'bifurcated hydroxamic acid' (diamide (1)), MGCD0103 (also under clinical study), sodium butyrate (short-chain fatty acid derivative, SCFA, with clinical use in IBD), and sodium valproate (SCFA). We studied the ability of these HDACis to modulate antigen- or agonist-induced contraction of isolated guinea-pig tracheal rings (GPTR).
Significant inhibition of the antigen-induced contraction of sensitized GPTR with the HDACis occurred after 1-3 h pre-incubation. Pre-incubation with HDACis for 2-6 h also significantly blocked GPTR contraction induced by histamine, 5-hydroxytryptamine (G-protein coupled receptor agonists) or low concentration (< 1 mM) of NaF (a non-selective G-protein activator), but minimally that by carbachol (though also GPCR-coupled). The inhibition by HDACis of agonist-induced contraction was not blocked by NO synthase inhibitors, suggesting that NO synthase induction is involved in the inhibition.
It is thought that histone acetylation is involved in the pathogenesis of asthma, chronic obstructive airways disease, and in the beneficial actions of corticosteroids [3] and theophylline in asthma, all of which may act through the influence of HDACis on gene expression. Most of the effects described appear to be via modulation of cell signaling, although those observed after extended preincubation with HDACis may involve modulation of gene expression.

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O28

Involvement of the histaminergic system in cytidine 5'-diphosphocholine-induced reversal of critical haemorrhagic hypotension in rats.

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Cytidine 5'-diphosphocholine (CDP-choline) is an endogenously formed mononucleotide, which exerts a variety of physiological effects by altering central cholinergic transmission [1]. Our previous studies demonstrate that CDP-choline administered intracerebroventricularly (icv) [2, 3] or intravenously [1] induces the reversal of haemorrhagic hypotension in rats, most probably as a result of the central cholinergic receptor stimulation. The present study demonstrates an influence of histamine receptor antagonists on central CDP-choline-mediated reversal of haemorrhagic hypotension. Experiments were carried out in ketamine/xylazine anaesthetised male Wistar rats subjected to a haemorrhagic hypotension of 20-25 mmHg. The mean arterial pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters blood flow were measured using the pressure transducer RMN-201 (Temed, Poland), the electrocardiograph Diascope 2 (Unitra Biazet, Poland) and the Transit Time Flowmeter type 700 (Hugo Sachs Elektronik, Germany), respectively. CDP-choline (2 μmol; icv) administered at 5 min of critical hypotension produced a long-lasting pressor effect with increases in MAP, HR, peripheral blood flow and a 100% survival at 2 h. The effects were almost completely blocked by H_1 receptor antagonist chlorpheniramine (50 nmol; icv). On the other hand, neither H_2 receptor blocker ranitidine (25 nmol; icv) nor H_3/H_4 receptor antagonist thioperamide (25 nmol; icv) influenced cardiovascular changes evoked by CDP-choline. In conclusion, there are interactions between the histaminergic and cholinergic systems in the central cardiovascular regulation in haemorrhagic hypotension in rats, and the results show an involvement of H_1 receptors in CDP-choline-induced resuscitating effect.

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O29

Diether (substituted)piperidine derivatives as a novel, active histamine H_3 receptor ligands.

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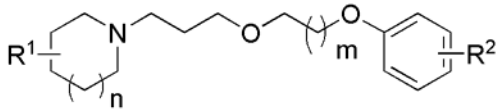
Histamine H_3 receptors are constitutively active G_i-protein coupled receptors mostly expressed in CNS. They have been described as presynaptically located auto- and heteroreceptors, that modulate the levels of histamine as well as that of other neurotransmitters such as: ACh, NA, 5-HT. Therefore, blockade of these receptors activity could be useful in the treatment of different CNS disorders, such as Alzheimer and Parkinson's diseases, dementia, ADHD, rhinitis or obesity [1].
The first known histamine H_3 receptor antagonists contained an imidazole group, which may be responsible for a number of side effects, due to its interaction with cytochrome P_{450}. One of the first successful imidazole replacements has been performed by piperidine moieties. According to the proposed pharmacophore for histamine H_3 receptors, the heterocyclic residue should be connected via the aliphatic linker with a polar moiety, connected itself by other linker with a lipophilic residue.
Paying attention to our previous investigations [2], and the results described in the literature, we obtained double-ether derivatives, to evaluate the influence of elongation of the alkyl spacer, as well as insertion of the second ether moiety, on histamine H_3 receptor binding properties.



n, m=1,2
R^1= -CH_3 (n=1)
R^2= -Cl, -OCH_3, -*t*-butyl

The novel compounds were evaluated for histamine H_3 receptor activity *in vitro* in a binding assay for the histamine hH_3 receptor stably expressed in CHO-K1 cells. Presented compounds show good to very good affinities.
Computational approaches allowed the prediction of physicochemical parameters such as logP and logD for the compounds examined [3].

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O30

Effective beta adrenergic inhibition of mast cell mediator release in the peripheral lung.

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Background: It is debated whether or not mast cell stabilization contributes to the anti-asthmatic effects of β_2 adrenergic agonists. The aim was to compare the effect of the β_2 agonist salbutamol with the standard mast cell stabilizer disodium cromoglycate (DSCG) in the peripheral lung.
Method: The study was performed in sensitized guinea pig lung parenchyma (GPLP), an *in vitro* model for antigen-induced contractions. The response is mediated predominantly by histamine, cysteinyl-leukotrienes (CysLTs) and prostanoids, thus similar to human airway responses. Preparations were set up in 5mL organ baths containing 37°C oxygenated Tyrode's solution. Mechanical responses were recorded isometrically and expressed as % of maximum induced contractions. Data are presented as mean ± s.e.m.
Results: Pretreatment with salbutamol (10-1000nM) and DSCG (100-1000μM) dose-dependently shifted the concentration-response curve of ovalbumin (OVA, 10-10,000ng/ml) to the right. Salbutamol 10nM (37 ± 7.2%; n=5; $p<0.01$) and DSCG 100μM (29 ± 10.1%; n=5; $p<0.001$) reduced the contractions compared to control (63 ± 5.0%; n=10) at OVA 1000 ng/ml. Both salbutamol 10nM (n=4) and DSCG 100μM (n=4) completely inhibited the release of CysLTs (<6.2 *vs* control 80.8 ± 26 pg/ml, $p<0.001$) and histamine (<5.0 *vs* control 24 ± 3.2 ng/ml, $p<0.001$), and reduced the release of thromboxane (TX) B_2 (salbutamol 448 ± 280 and DSCG 370 ± 89 *vs* control 2788 ± 700 pg/ml, $p<0.001$) in the organ bath at OVA 1000 ng/ml. Salbutamol (0.0001-1000μM) dose-dependently relaxed LTD_4 (10nM) precontracted GPLP (n=6; $p<0.001$).
Conclusions: At doses where salbutamol caused minor bronchodilation, the release of histamine, LTE_4 and TXB_2 was strongly affected. As DSCG caused similar inhibition of the functional response and mediator release, both findings together support the view that inhibition of mast cell mediator release is likely to contribute to the overall effects of β_2 agonists on peripheral airways.

O31

Histamine H_1 receptor stimulation on keratinocytes leads to increased MMP-9 production.

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Background: Histamine is an important mediator in allergic skin diseases and can be found at increased concentrations e.g. in lesions of atopic dermatitis. The effect of histamine on migration and function of cells relevant in allergic skin diseases such as keratinocytes is not well understood.
Objectives: The aim of this study was to investigate the effect of histamine on MMP-9 production by human primary keratinocytes (KCs), since MMP-9 is an important mediator of tissue remodelling in skin inflammation.
Methods: MMP-9 production by cultured KC and skin biopsies was measured by ELISA and gelatine zymography. The biological effects of MMP-9 production were determined by immunohistological staining of type IV collagen in skin sections and trans-basement membrane migration assays.
Results: Histamine induced dose-dependent up-regulation of MMP-9 in KCs and in punch biopsies of human skin. The histamine H_1 receptor (H_1R) agonist β-histine – but not agonists for H_2R, H_3R and H_4R – induced MMP-9, whereas the H_1R antagonist clemastine blocked the effect in dose-dependent manner. Immunohistological staining showed that histamine-induced MMP-9 lead to destruction of type IV collagen at the basement membrane of healthy skin. In a co-culture system of KCs and T cells, migration of T cells through an artificial basement membrane was increased after histamine stimulation of KCs.
In conclusion, the stimulation of H_1R on KCs lead to increased levels of MMP-9 and, thereby, facilitated the migration of T cells across the basement membrane. These findings may represent a new mechanism by which histamine contributes to the pathology of allergic skin diseases.

O32

Mast cell-derived histamine: in vivo and in vitro effects on tumor growth.

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Despite the fact that accumulation of mast cells is frequently observed in the environment of tumor types, their role in tumor progression is not characterized in detail. It is known that the majority of mast cell-derived factors can promote, but others can suppress, tumor progression. The mast cells can be the main source of histamine in the local environment of malignant growth, which is also a relevant player in tumor growth.
To determine the role of mast cell-derived histamine in tumor growth, in our studies wild-type and HDC-/- bone marrow – derived mast cells were applied from Balb/c mice. As tumor cells, mammary adenocarcinoma (LM2) and dermatofibrosarcoma (DFS) cell lines were used. The *in vivo* growth was measured following the subcutan inoculation of suspensions of tumor cells and mast cells on the flanks of mice. The interaction between mast cells and tumor cells was examined in co-culture system *in vitro*. In the presence of mast cells, the altered chemotactic activity of LM2 adenocarcinoma cells was evaluated by a fibronectin-coated haptotaxis assay.
Our data show that in our model the exogenic mast cells are able to promote the growth of tumor grafts, especially the histamine-producing, namely wild-type mast cells. In the background of this phenomenon, a Th2-polarized local immune response, and altered expression of angiogenic and invasion markers was found in the presence of wild-type mast cells. In respect of the influence of migratory potential of cells, it was observed that the lack of histamine has differential importance in the case of mast cells and tumor cells.

O33

IMI – a new opportunity for research funding in Europe

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The largest European public-private partnership in biomedical research, the Innovative Medicine Initiative (IMI) starts this year. This is a unique pan-European research and development initiative with strategic focus of strengthening the competitiveness of European research with focus on drug development. The aim is to stimulate faster discovery and development of safer and more effective medicines for patients. The founding organisations are the European Federation of Pharmaceutical Industries and Associations (EFPIA) and the European Commission (EC). The partnership is a €2 billion joint venture, which is set up to run over the next ten years. The funding system is balanced 1:1, the EC will contribute public funding and the industry will contribute in-kind, for instance by providing access to specialized expertise, platform approaches and other means of support. Public consortia made up of universities, hospitals, regulatory authorities, small- and medium-sized biopharmaceutical and healthcare companies (SMEs), and patient organizations will, on a competitive basis, be able to apply for funding. Approved applications will be matched by equal in-kind resources from the EFPIA members. The presentation will describe the strategic research agenda of IMI, the selection process for grants and the first calls that are to published late in April.

Poster Communications

P1

Pheniramines and Oxidative Burst of Blood Phagocytes During Ischaemia/Reperfusion

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Pheniramines belong to the group of potent H_1-antihistamines. They were found to possess antiradical activity which (i) depended on physico-chemical parameters and (ii) focused selectively on extracellularly formed reactive oxygen metabolites (1, 2).
In this work, we studied the oxidative burst of rat blood phagocytes exposed to pheniramines during mesenteric ischaemia (60 min)/reperfusion (30 min) injury. Concentration of oxidants was measured by luminol-enhanced chemiluminescence, using 0.25 µl samples of whole blood and a luminometer LM-01T Immunotech. Both *in vivo* and *in vitro* effects were analysed – i.e. pheniramines were administered 30 min before occlusion of the superior mesenteric artery and immediately before reperfusion in the total dose 2x10 mg/kg i.p., and they were added after reperfusion to the blood of non-medicated rats in the concentration range 1-100 µmol/L.
Whole-blood phagocyte count increased after reperfusion from 12.4 (sham-operated) to 14.1 (no medication), 15.1 (pheniramine), 16.5 (chlorpheniramine) and 20.2 (brompheniramine) x 10^3/µL. Compared to sham-operated rats, ischaemia/reperfusion increased chemiluminescence stimulated with phorbolmyristate acetate by 182%. Administration of pheniramines resulted in a less pronounced chemiluminescence increase - by 72% (pheniramine), 94% (chlorpheniramine) and 1% (brompheniramine). Under *in vitro* conditions, chlor- and brompheniramine significantly decreased chemiluminescence at concentrations of 50 and 100 µmol/L, while pheniramine was ineffective.
The results have confirmed the *in vivo* effectiveness of pheniramines and their ability to reduce activation of phagocytes during ischaemia/reperfusion.

1. Jančinová V. et al. Inflamm Res 2006;55:S85-6
2. Jančinová V. et al. Neuroendocrinol Lett 2006;27:141-3
Supported by grants VEGA 2/7019/27, VEGA 2/5009/25 and APVV SK-CZ-0114-07

P 2

Protective effect of Pheniramines against mesenteric ischaemia/reperfusion-induced injury.

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Pheniramines – a group of H_1 antihistamines, were found to decrease radical formation by neutrophils activated by inflammation. Halogen substitution in the antihistamine molecule may potentiate its antiradical activity. This may prove beneficial as it could reduce tissue damage. The aim of our work was to study the possible protective effect of pheniramines in the rat model of mesenteric ischaemia/reperfusion (I/R) induced injury.
The extent of intestinal damage caused by I/R was recorded, the activity of myeloperoxidase (MPO) was measured, and evidence of increased free radical production was assessed by the chemiluminescence (CL) response of the ileal samples. The effect of H_1 antihistamines pheniramine, chlorpheniramine and brompheniramine administered at the dose of 10 mg kg^{-1} twice i.p. was studied *in vivo*.
I/R induced pronounced haemorrhagic intestinal injury. All pheniramines reduced significantly the extent of damage. A reperfusion-induced MPO increase was also inhibited by all pheniramines. Comparing to sham-operated rats, the CL response of the ileal samples increased after I/R with a further increase caused by pheniramines, probably in association with neutrophil count in whole blood, which was increased in all groups after reperfusion.
The results obtained showed a protective effect of all pheniramines studied on mesenteric I/R. Contrary to the effect of halogenisation on pheniramine activity *in vitro,* with the potency order pheniramine<chlorpheniramine< brompheniramine (Jančinová et al., 2006), there were no significant differences in their *in vivo* effects.

The work was partially supported by VEGA grant No 2/5009/25 and 2/7019/27.
Jančinová V. et al. Inflamm Res 2006;55: S 85-6

P3

Plasma Histamine-Levels in Organic Farming Piglets: Differences Due To Immunization and Weaning.

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During weaning piglets are under strain from the loss of their dam, the change in feed, and from a new microbiological environment. These changes are strong stressors. Performance and health status of organic piglets is often not satisfying even if piglets are weaned later (at least 40 days) than piglets from conventional rearing. Therefore, it was the aim of the present study to investigate whether a prolonged suckling period of 63 days results in better performance compared to weaning at day 42, and how much the plasma histamine-level is influenced by this.
36 sows were allotted to a 2 x 2 Latin Square design with the treatments weaning (early [day 42] and late [day 63]) and, due to other reasons, vaccination (early [day 42] and late [day 63]) of the piglets. Secondary vaccination was performed 21 days later. Blood was sampled on day 42, 49, 63, 70, 84 and 91. Plasma histamine was analysed by HPLC. Time of blood sampling procedure was recorded.
Time of blood sampling did not differ at any sampling day between early and late weaned piglets. Independently from time-point of weaning, plasma histamine increased in early vaccinated piglets after day 49 (from 28 to 47 nM; $n = 60 - 70$; $P < 0.01$; Repeated Measures ANOVA). This was not the case in late vaccinated piglets. In those piglets, differences in plasma histamine were found between early and late weaned piglets on days 84 (35.8 vs. 46.8 nM) and 91 (36.4 vs. 47.3 nM; medians; $n = 53 - 68$; $P < 0.05$; Mann-Whitney rank sum test).
The influence of different blood sampling stress on plasma histamine-levels can be excluded. Different courses in the levels of plasma histamine between early and late vaccinated piglets led to the conclusion that piglets react timepoint-dependently on vaccinations. In contrast, weaning time-point did not seem to have an influence on plasma histamine in piglets, at least in early vaccinated individuals.
This work was supported by the Bundesprogramm Ökologischer Landbau, Project No. 03oe378.

P4

The selective histamine H_4 receptor antagonist JNJ7777120 exhibit antipruritic efficacy in two murine models of contact dermatitis, but does not effect inflammation.

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JNJ7777120 is a highly selective antagonist of the histamine H_4 receptor (H_4R). Early findings indicate the H_4R as a novel therapeutic target for the treatment of inflammatory and immune disorders. Effects of JNJ7777120 were tested in two different hapten-induced allergic dermatitis models in mice. Repeated topical exposure to the haptens 2,4-dinitrochlorobenzene and toluene-2,4-diisocyanate elicits a specific T cell-dependent immune response with signs of inflammation and pruritus. 2,4-Dinitrochlorobenzene induces a Th1 dominated inflammation, whereas toluene-2,4-diisocyanate causes a Th2 response. In both models JNJ7777120 (15 mg/kg i.p.) administered 2 h and 30 min before and 1 h after challenge did not reduce hapten-induced inflammatory response (ear swelling) determined 24 h after challenge. By contrast, systemic treatment with JNJ7777120 (1.25 to 15 mg/kg i.p.) 30 min before challenge reduced hapten-induced scratching in a dose-dependent manner. In comparison to H_4R antagonism, antipruritic potential of the H_1 receptor antagonist cetirizine (15 mg/kg) and the H_2 receptor antagonist ranitidine (15 mg/kg) were tested. Scratching was also suppressed by treatment with cetirizin, whereas ranitidine failed to inhibit pruritus. A combination of H_1 and H_4 receptor antagonists resulted in the strongest inhibition of scratching behaviour. These results indicate that H_4R antagonism fails to reduce the allergic inflammatory response but strongly inhibits allergen-induced itch.

P5

Expression and function of histamine H_4 receptor in human $CD4^+$ T cells.

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Background: T lymphocytes are present in increased numbers in allergic skin diseases. Histamine, released from effector cells during inflammation, can influence T cell reactions via H_1R and H_2R. Histamine H_4 receptor (H_4R) is the most recently identified histamine receptor and is also expressed in human $CD4^+$ T cells, however, its regulation and function remain less clear.
Objectives: The distribution and regulation of the H_4R in naïve T cells, Th1 polarized and Th2 polarized T cells were assessed. Furthermore, *in vitro* effects of histamine and H_4R agonists on these cells were investigated.
Methods: Quantitative real time LightCycler RT-PCR was used to investigate the regulation of H_4R expression in the different subtypes of T cells. The cell surface expression of H_4R was measured by FACS analysis and Western blot. To elucidate effects of H_4R on signal transduction, an electrophoretic mobility shift assay (EMSA) was applied.
Results: H_4R mRNA and protein could be demonstrated in $CD4^+$ T cells, Th1 and Th2 cells. IL-4, a Th2 cytokine, up-regulated H_4R mRNA in $CD4^+$ T cells. The H_4R is up-regulated in Th2 cells as compared with Th1 cells at the mRNA and protein levels. H_4R stimulation induced the transcription factor AP-1 in Th2 cells. DNA binding of AP-1 was completely inhibited by the H_4R antagonist JNJ7777120.
Conclusion: H_4R is up-regulated in Th2 cells as compared with Th1 cells. The stimulation of H_4R activates the transcription factor AP-1 in Th2 cells. Further studies of downstream events of AP-1 (e.g. cytokine and chemokine release) are currently being performed to elucidate the role of participation of H_4R in allergic reaction.

P6

Histamine receptors genes knockout mice and their phenotypes

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Background: The role of histamine as a neurotransmitter has been demonstrated for over 20 years. The histamine receptor subtypes (H_1, H_2, H_3, and H_4) mediate these neuronal actions. There are many physiological roles of histamine in the brain such as sleep-awake cycle, locomotors activity, appetite and drinking, convulsion and learning/memory. These functions have been examined using histamine antagonists/agonists and single gene receptor knockout mice. The phenotypes of single gene knockout mice are characteristic of each histamine receptor. H_1 receptor gene deficient mice significantly increased the ratio of ambulation during the light period to the total ambulation for 24 hr in an accustomed environment, and the H_3-deleted mice showed a decrease in overall locomotion, wheel-running behavior, and body temperature during the dark phase but maintained normal circadian rhythm. H_2 receptor-deficient mice exhibited a marked hypertrophy with enlarged folds in gastric mucosa with elevated levels of serum gastrin. However, it is still unclear whether the deficiency of multiple histamine receptors has any effects on maintenance of life and fertility.
Aim: We make multiple histamine receptors genes knockout mice to clarify the effects of multiple histamine receptor gene deletions on phenotypes.
Methods: An H_1 receptor knockout female mouse was mated with a male H_2 receptor knockout mouse. The male H_1, H_2 double receptor knockout offspring was then mated with a female H_3 receptor knock out mouse (a gift from Dr. Lovenberg).
Results: H_1, H_2, H_3 triple histamine receptors gene deficient mice (TKO) and H_1, H_3 double histamine receptors knockout mice (H_1, H_3-DKO) were born and were viable. However, H_2 receptor multiple histamine receptor gene knockout mice were difficult to make for unknown reasons.

P 7

Trafficking of Histamine H_2 Receptor, Phosphorylation Dependent and Independent Regulation of H_2R by GRK2.

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Histamine H_2 receptors (H_2R) belong to the large family of G-protein coupled receptors (GPCRs). By coupling to Gs protein, H_2R stimulation triggers adenylyl cyclase activation, and in turn rapid cAMP accumulation. Following H_2R phosphorylation and desensitization by GRK2, H_2R are internalized and recycled back to the cell membrane. It is widely assumed that specific inhibition of GPCRs response occurs by β-arrestin binding to the phosphorylated receptor. In this context, GRK major function is to enable β-arrestin binding to the receptor and uncoupling from the heterotrimeric G protein.
In the present study, we investigated whether H_2R phosphorylation was necessary for the desensitization and/or internalization/resensitization of the H_2R. Therefore the effect of GRK2K220R (a phosphorylation-inactive mutant with disrupted kinase activity) was evaluated on these processes in COS7-H_2R and U937 transfected cells.
In COS7-H_2R cells, transfection with either GRK2K220R mutant or GRK2 wild type, led to a 30% decrease in cAMP response to amthamine due to enhanced H_2R desensitization. When H_2R internalization and recycling were determined by binding assays, we found that GRK2K220R dampened H_2R internalization and therefore resensitization, therefore behaving as the GRK2 antisense construct. Similar results were obtained in U937 cells stably transfected with the GRK2K220R mutant.
These findings support that receptor phosphorylation results crucial for H_2R internalization and resensitization. However, kinase activity is not necessary to achieve H_2R desensitization. Furthermore, the GRK2K220R mutant attenuates H_2R signaling to a similar extent as the GRK2 wild type kinase, revealing a phosphorylation-independent mechanism of H_2R regulation by GRK2.

P8

The First Panel of Immunological Probes for Human H_3 Histamine Receptor Isoforms.

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The existence of multiple H_3 receptor isoform mRNAs has opened up possibilities to account for the pharmacological heterogeneity in H_3 receptors, within and across species, which has long been recognised. The reasons for this heterogeneity are complex and not fully understood. In all species tested so far, the full length H_3 receptor encodes a polypeptide of 445 amino acids (predicted polypeptide Mr 47,000). Shorter human isoforms (up to 20) with deletions predominantly in the third intracellular loop domain have also been identified (predicted polypeptide M_r range 20,000 - 45,000). While the full length clone is found in most abundance in the CNS in all species studied so far, there is regional variation in the distribution of the different isoform mRNAs. This has given rise to speculation that H_3 heterogeneity could underlie different activities and functions of H_3 receptors in specific brain areas. Furthermore, we have growing evidence that heteroligomerisation of H_3 isoforms may occur and yield a novel regulatory mechanism (Bakker *et al.,* 2006)
In order to define the importance of human H_3 receptor heterogeneity, specific isoform specific immunological probes are required. Our laboratory has developed the first panel of anti-human H_3 receptor isoform-directed antibodies. Two antibodies (termed anti-pan H_3R) were raised against human H_3 receptor sequences common to most human and rodent isoforms: anti-H_3 (346-358) and anti-H_3 (175-187), respectively (Chazot *et al*, 2001; Cannon *et al.*, 2007; Hann et al., unpublished). Recently, we have generated an anti-$hH_{3\,(445)}$ specific antibody (Bakker et al., 2006), and an anti-$hH_{3\,(329)}$ specific antibody (Shenton et al., unpublished). The human $H_{3\,(220)}$ (isoform 5) has a large 170-306 deletion plus a frame shift and a novel stop codon, yielding a unique C terminal sequence, CRRPRPRWRSA. We have generated a novel anti-$H_{3\,(220)}$ antibody raised to this sequence, which identified a major specific species (M_r 72,000) in human putamen on immunoblotting, which was blocked by preincubation with the respective peptide. The size of this immunoreactive species corresponds to a putative $hH_{3\,(445)/}$ $hH_{3\,(220)}$ heterodimer, co-incident with one of the species labeled by both the pan and anti- $hH_{3\,(445)}$ specific antibodies. We are currently confirming isoform specificity of the $hH_{3\,(220)}$ directed antibody versus individual recombinant human hH_3 clones expressed in HEK 293 cells.

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P9

Characterization of Histamine-Induced Relaxation in Pre-Contracted Rat Aorta.

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We studied modulation of histamine-induced relaxation of isolated rat aortic rings with histamine H_1, H_2 and H_3 receptor antagonists.
Thoracic rat aorta rings were placed in organ baths filled with oxygenated Krebs-Henseleit solution. The presence of functional endothelium was assessed in all preparations by determining the ability of acetylcholine (1 μM) to induce more than 50 % relaxation of rings pre-contracted with noradrenaline (0.1 μM). Rings were then incubated for 30 minutes with selective H_1-, H_2- and H_3-receptor antagonists: chloropyramine, cimetidine and thioperamide, respectively. After contraction with 0.1 μM noradrenaline reached a steady state, increasing concentrations of histamine were added cumulatively (ranging from 1 nM to 0.1 mM). Responses were measured using an isometric mechano-electrical transducer and relaxation was expressed as a percentage of noradrenaline pre-contracted aortic rings values.
Histamine relaxed aortic rings in a concentration-dependent manner in all studied groups. Maximum relaxation response in the control group was 88.90 ± 1.99 %. In H_1-antagonism experiments, relaxation decreased with increasing chloropyramine concentration (0.1 μM, 1 μM, 10 μM): 57.52 ± 1.71 %, 42.63 ± 3.16 %, 38.39 ± 1.91 %, respectively. Similarly, in H_2-antagonism experiments, relaxation decreased with cimetidine (1 μM, 10 μM): 62.46 ± 2.20 %, 66.15 ± 3.72 %, respectively. In H_3-antagonism experiments (1 μM thioperamide) relaxation response was unaltered compared to the control group.
In conclusion, both H_1-receptor blockade with chloropyramine and H_2-receptor blockade with cimetidine caused a partial blockade of the relaxing effect of histamine in the thoracic rat aorta. However, H_3-receptor blockade with thioperamide did not influence the histamine-induced vasorelaxation. These results show that vasorelaxation in rat aorta induced by histamine mainly depends on its action on H_1 and H_2 receptors.

P10

Famotidine Increases Bone Volume in the Late Stage of Orthodontic Tooth Movements in Rats

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Bone modelling is the key process in orthodontic tooth movement (OTM). On the pressure side of the loaded tooth, the alveolar bone is resorbed by osteoclasts and on the tension side, new bone is formed by osteoblasts. Many chemical messengers are involved in this processes, among them is histamine. It decreases OTM in its late stage via H_2 receptors (1).
The aim of this study was to determine the influence of famotidine, a H_2 antagonist on osteoblasts and osteoclasts after 42 days of OTM in rats.
Male Wistar rats (n=30) were divided into three groups: control animals (n=10) without appliance, and test animals in which a super-elastic closed coil spring was applied between the first left maxillary molar and the incisors and treated daily with famotidine (n=10 – famotidine group) or with saline (n=10 – appliance only group). All animals were sacrificed on day 42 and tissue samples were prepared for histological and histomorphometrical analysis. Alveolar bone volume, osteoblast and osteoclast volume were determined histomorphometrically.
Alveolar bone volume was significantly higher in the famotidine group compared to the appliance only group ($p<0.001$). Osteoclast volume was significantly lower in the famotidine group compared to the appliance only group ($p<0.05$). Osteoblast volume was significantly higher in the famotidine group compared to the appliance only group ($p<0.05$).
This suggests that histamine increases osteoclastic bone resorption and osteoblastic bone formation via H_2 receptors in the late stage of OTM in rats.

1. Sprogar Š, Križnar I, Drevenšek M, Vaupotič T, Drevenšek G. Famotidine, a H_2 receptor antagonist, decreases the late phase of orthodontic tooth movement in rats. Inflamm Res 2008, in press.

P11

Histamine-releasing factor in human choroid plexus.

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The choroid plexus (CP) plays a pivotal role in the function of the central nervous system by production of cerebrospinal fluid (CSF) and secretion of numerous neuropeptides, growth factors and cytokines. Recently, a prominent histidine decarboxylase mRNA expression essential for histamine production was found. The aim of the present study was to examine the ultrastructure of CP, the number of mast cells (as a source of histamine), and the expression of mRNA and immunolocalization of histamine-releasing factor (HRF) in CP of patients with paraneoplastic cerebellar degenerations (PCD) - non metastatic complication as a remote effect of neoplasms located in the peripheral organs.
The study was performed on human brain samples obtained following autopsy. For *in situ* hybridization and for immunochemistry respectively, HRF nucleotide probes and specific antibodies generated against mast cells tryptase, HRF protein, histamine receptors H_1, H_2 and H_4 as well as against the "paraneoplastic" Yo and Hu antigens were used. The results showed that morphological lesions of CP were localized in the epithelium, basement membrane and vascularized matrix. In the epithelium, 74% of cells were overloaded with lipofuscin. The basement membrane of epithelium was irregular and thick. In the matrix, numerous deposits of fibres were present. The HRF mRNA expression and HRF protein were found in CP epithelial cells and both of them were more prominent in the well preserved cells than in those overloaded with lipofuscin. The CP tryptase positive mast cells were few in number. In conclusion, results of the present study suggest that histamine metabolism in the CP of patients with the PCD is located in the CP epithelial cells but not concomitant with mast cells infiltration of CP, and that the lesions of CP may affect the production and release of this monoamine to CSF.

P12

Uptake of L-histidine and histamine biosynthesis at the blood-brain barrier

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OBJECTIVE AND DESIGN: To elucidate the functional importance of histamine at the blood-brain barrier (BBB), we discuss recent findings regarding the transport characteristics and metabolism of L-histidine, a precursor of histamine, in cultured rat brain microvascular endothelial cells (BMECs) which are major structural components of the BBB. Moreover, we study whether histamine formed in BMECs increases paracellular permeability through tight junctions in BBB.
MATERIAL: Male, 3-week-old Wistar rats were used. For in vitro studies, rat BMEC were isolated from rat brains, and subculture cells were grown on collagen-coated culture flask and slide, and on transwell membrane.
METHODS: L-Histidine uptake, HDC assay, immunofluorescence analysis, expression of HDC mRNA by RT-PCR and transport clearance of fluorescein were performed in rat BMECs.
RESULTS AND DISCUSSION: L-Histidine was uptaken into rat BMECs via both Na+-dependent system N and Na+-independent system L transporters. Zinc ion had an enhancing effect on the BBB transport of L-histidine. L-Histidine is biotransformed to histamine by L-histidine decarboxylase (HDC). The presence of HDC protein and the expression of HDC mRNA were confirmed in rat BMECs, and the HDC activity of the BMECs was estimated to be 0.14 ± 0.05 pmol/mg protein/min. These findings indicated that L-histidine uptaken into rat BMECs was shown to be converted to histamine, suggesting that HDC may play an important role in the regulation of paracellular permeability through tight junctions in BBB. However, in our experiment, although L-histidine was added into the medium, transport clearance of fluorescein was not increased. Since the expression of monoamine oxidase-B mRNA was also confirmed in rat BMECs, histamine turnover may be so fast at BBB.

P13

Selective effects of dexchlorpheniramine on stages of human information processing.

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Centrally acting antihistamines can impair human information processing. A previous study in healthy volunteers has shown that these drugs specifically affect psychomotor performance, while having no specific effects on memory and learning (Van Ruitenbeek et al., in press). The mechanisms underlying these effects are still unclear however. The aim of the present study was therefore to further elucidate subprocesses involved in psychomotor performance that may be affected by antihistamines, by studying the interactions of dexchlorpheniramine with specific task manipulations in a choice reaction time task. Task demands were increased at the level of perceptual subprocesses by degrading stimulus quality and at the level of motor subprocesses by increasing response complexity.
A total of 18 healthy volunteers (9 female) aged between 18 and 45 years, participated in a 3-way, double blind, cross-over design experiment. Treatments were single oral doses of dexchlorpheniramine 4mg, lorazepam 1mg and placebo. Behavioral effects were assessed using a choice reaction time task and effects on brain activity were assessed by Event Related Potentials (ERPs).
Results showed that dexchlorpheniramine significantly slowed reaction times, but there were no significant interactions with task manipulations. There was, however, a significant interaction with stimulus degradation as measured by ERPs, suggesting specific effects on feature extraction processes.
Lorazepam slowed reaction times and interacted with perceptual as well as motor manipulations, as shown by effects on reaction times and ERPs, suggesting that the effects of lorazepam are relatively nonspecific.

Van Ruitenbeek, P., Vermeeren, A., Smulders, F., Sambeth, A., Riedel, W. J. Histamine H_1-receptor blockade in humans affects psychomotor performance but not memory. Journal of Psychopharmacology. In Press

P14

Nature of histamine transport in neonatal rat cultured astrocytes.

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Astrocytes, which express numerous transporters on their membranes, play a prominent role in uptake of various neurotransmitters. They have been shown to take part in the reuptake as well as in the metabolism of histamine, however, a specific histamine transporter, whether on astrocytes or anywhere else has yet to be been elucidated. It has been proven that one of organic cationic transporters (OCT3) is involved in handling of monoamines across biological membranes.
In the present work, we wanted to determine the nature of histamine transport into cultures astrocytes and to establish whether OCTs might take part in this process.
Histamine was taken up into neonatal rat astrocytes in a time- and concentration-dependent manner by two independent processes – a passive electrodiffusion and additional carrier-operated process. A passive electrodiffusion represented 59% of the total transport and was dependent on concentration gradient of histamine only. A carrier-operated uptake, appearing at 37 □C, revealed a single carrier system with a Michaelis-Menten's constant (Km) of 3.5 +/- 0.8 microM and a maximal uptake rate (Vmax) of 7.9 +/- 0.3 pmol/mg protein/min. In order to assess the role of OCTs in histamine transport, we observed the influence of a specific OCT inhibitor decynium 22 on histamine uptake into neonatal rat cultured astrocytes. Decynium 22, in a concentration range 0.1–50 μM, modestly (up to 28%) inhibited histamine uptake into cultured astrocytes compared to controls however, this inhibition did not reach statistical significance, $p = 0.39$, ANOVA).
Our results suggest that astrocytes play an important role in the inactivation of histamine. Histamine is transported into astrocytes by a passive electrodiffusion and an active transport process, which is operated by yet not identified carrier, and not by one of the OCTs.

P15

Histamine 3 Receptor Inverse Agonists for the Control of Food Intake and Beyond

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Withdrawn

P16

Histamine H_4 receptors are not involved in the regulation of cholinergic neurotransmission in the rat duodenum.

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The recently cloned histamine H_4 receptor (H_4R) was found to play a role in inflammatory and immune responses. Recently, immunohistochemical data have provided evidence that the H_4R is also expressed in myenteric neurons of the rodent gastrointestinal tract, suggesting a role for this receptor in the regulation of intestinal peristalsis (1). In the present study we examined whether H_4Rs have a role in the regulation of intestinal neurotransmission, by testing selective H_4R ligands in the isolated rat duodenum.
Twitch responses of longitudinal muscle strips to electrical field stimulation were abolished by atropine (1 μM) and partially reduced by hexamethonium (10 μM), suggesting that pre- and postganglionic cholinergic neurons were activated. Under these experimental conditions, the H_4R agonist VUF8430 (2) and histamine, in the presence of H_1-, H_2- and H_3-receptor blockade, did not change electrically-evoked contractions. Similarly ineffective were the selective H_4R antagonists VUF10148 and VUF10214 (3), when tested at 10 nM and 100 nM. At higher concentrations (1-10 μM), these compounds evoked a dose-dependent inhibition of electrically evoked contractions, which, however, was mimicked by VUF10181, a chemically related analog, endowed with a 1000-fold lower affinity at H_4R (3).
In conclusion, functional experiments on isolated rat duodenum did not unravel any role of H_4Rs in the control of intestinal neurotransmission.

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(3) Coruzzi, G et al. XXXVI EHRS Meeting, Florence, May 9-12th 2007, p.106

P17

H3 receptor stimulation decreases gamma oscillations in the hippocampus.

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The histamine system is involved in arousal and cognition. Administration of histamine H_3 receptor antagonists can enhance performance in cognitive tasks. Interestingly histamine regulates some of the physiological activity patterns believed to underlie cognitive processes such as local field-potential oscillations. Moreover, histamine H_3 receptors have prominent expression in the hippocampus, the key area for learning and memory. At the cellular level, H_3 receptors are predominantly expressed presynaptically and are Gi coupled. Through inhibition of release they act as autoreceptors, which is a mechanism by which histamine can influence network activity. Gamma frequency oscillations (typically 30 - 100 Hz) are seen in several cortical regions during cognitive tasks such as memory encoding, and has been proposed to have an important role in information processing. The modulation of this type of oscillations may be one way in which histamine influences cognition. We have investigated the effects of H_3 receptor agonists/antagonists on kainate-induced gamma oscillations in an *in vitro* hippocampus slice preparation.
Local field potential oscillations were induced by treating the slices with 100 nm kainate. Field potential recordings were recorded in the CA3 area at regular intervals and power spectral analysis was performed. Power in the 20 to 80 Hz band was quantified. We found that the H_3 receptor agonist R-α-methylhistamine inhibits oscillatory activity compared to baseline and that this effect can be reversed by the H_3 receptor antagonist clobenpropit.

P18

Orexin type 1 receptor antagonist SB 334867 inhibits central histamine-induced resuscitating effect in rats subjected to haemorrhagic shock.

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Histamine, acting centrally as a neurotransmitter, induces the reversal of critical haemorrhagic hypotension in rats [1]. Our previous studies demonstrate that orexin A given intracerebroventricularly (icv) to haemorrhage-shocked rats evokes a similar effect as a result of the sympathetic system activation [2]. There are functional interactions between orexinergic and histaminergic neurones in many central nervous system functions, including regulation of sleep and feeding [3] and, therefore, the purpose of the present study was to examine the influence of orexin type 1 (OX1) receptor blockade on central histamine-induced cardiovascular effects in rats. Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats. Mean arterial pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters blood flow were measured using the pressure transducer RMN-201 (Temed, Poland), the electrocardiograph Diascope 2 (Unitra Biazet, Poland) and the Transit Time Flowmeter type 700 (Hugo Sachs Elektronik, Germany), respectively. Irreversible haemorrhagic shock was induced by intermittent bleeding from the right jugular vein over a period of 15-25 min, until MAP stabilised at 20-25 mmHg. Histamine (100 nmol; icv) administered to haemorrhage-shocked rats evoked long-lasting rises in MAP and HR, with a subsequent increase in peripheral blood flow and a 100% survival of 2 h. The selective non-peptide OX1 receptor antagonist SB 334867 (5 mg/kg; intraperitoneally) inhibited histamine-induced haemodynamic effects, however, without influence on the survival rate at 2 h. In conclusion, there are functional interactions between orexinergic and histaminergic systems in the central cardiovascular regulation in critical haemorrhagic hypotension in rats.

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P19

Interactions between the serotonergic and histaminergic systems in the central cardiovascular regulation in haemorrhage-shocked rats: involvement of 5-HT1A receptors.

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Centrally acting histamine evokes the reversal of critical haemorrhagic hypotension in rats [1]. Similarly, serotonin acting via central 5-HT_{1A} receptors induces the pressor effect in haemorrhage-shocked rats [2]. Since our previous studies revealed the inhibitory action of H_1 receptor antagonist chlorpheniramine on central serotonin-induced pressor effect [3], the aim of the present study was to examine a possible involvement of 5-HT_{1A} receptors in histamine-mediated resuscitation. Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats weighing 230-260 g. Mean arterial pressure (MAP) and heart rate (HR) were measured using the pressure transducer RMN-201 (Temed, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Poland), respectively. Irreversible haemorrhagic shock was induced by intermittent blood withdrawal from the catheter inserted into the right jugular vein over a period of 15-25 min, until MAP stabilised at 20-25 mmHg. The bleeding volume for induction of critical hypotension was 2.53 ± 0.28 ml/100 g b.w. Histamine (100 nmol; intracerebroventricularly [icv]) administered to haemorrhage-shocked rats evoked long-lasting rises in MAP and HR, with a 100% survival of 2 h. Pre-treatment with a selective 5-HT_{1A} receptor antagonist, WAY 100635 (30 nmol; icv), partially inhibited histamine-induced action. In addition, the pressor and tachycardic effects of 5-HT_{1A} receptor agonist 8-OH-DPAT (10 nmol; icv) were diminished after pre-treatment with chlorpheniramine (50 nmol; icv). In conclusion, there are bi-directional interactions between the histaminergic and serotonergic systems in the central cardiovascular regulation in critical hypotension in rats, and H_1 and 5-HT_{1A} receptors are involved.

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P20

Regulation of neurotrophin-3 synthesis in astrocytes by histamine.

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Withdrawn

P21

Differential effects of potassium channel blockers on anti-IgE-stimulated histamine release from human basophils.

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An enhanced cytosolic Ca^{2+} concentration is thought to crucially promote mast cell and basophil mediator release to a variety of stimuli. Previous studies have suggested that extracellular calcium influx may be affected by the involvement of voltage-dependent K^+ channels in these cells. In light of this, we investigated the effects of different selective and non-selective K^+ channel blockers on IgE-dependent histamine release from basophils. Human basophils were enriched by Ficoll-density centrifugation and further purified by negative selection with magnetic cell sorting in some experiments. Basophils from a range of healthy donors were treated with the drugs (apamin, barium chloride, clotrimazole, iberiotoxin, tetraethylammonium chloride and 4-aminopyridine) for 15 min before stimulation with anti-IgE. After 30 min reactions were terminated and histamine releases were assessed spectrofluorometrically. None of the drugs tested produced any significant inhibition of IgE-dependent degranulation, with exception of 4-aminopyridine which reduced histamine release by 53.1 ± 4.2 % at the 10 mM and without attenuation in viability. In contrast, barium chloride potentiated IgE-induced histamine release above 10 mM and additionally caused substantial release of the amine in the absence of anti-IgE. Overall, these data suggest a lack of voltage-dependent potassium channel input in human basophil degranulation processes. However, given the actions of barium chloride, other potassium channels, such as inward rectifiers, may still be involved.

P22

Involvement of Organic Cation Transporter in Histamine Uptake into Human Vascular Endothelial Cells.

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Endothelial cells are the first major cell type to encounter histamine upon its release into the blood. Elevation of plasma histamine level (≥10 nM) is associated with pathological symptoms but significantly lower plasma histamine levels are harmful during pregnancy. Therefore, it is important to better elucidate the capacity and strategies of endothelium for elimination of increased histamine levels from the circulation. We have already shown that endothelial cells participate in the inactivation process of histamine by taking it up and that the organic cation transporter 3 (OCT3) might be involved in histamine transport. The objective of the present study was to provide additional evidence for OCTs involvement in histamine uptake into endothelial cells at two separate incubation times: 5 and 45 minutes.

We used the model system of cultured human umbilical vein endothelial cells (HUVEC) and followed [^{3}H]-histamine uptake in a serum-free buffer. Specific [^{3}H]-histamine uptake was saturable at both time points, but with lower K_m and capacity at 5 minutes. OCTs inhibitor decynium-22 (100 nM) and H_1 receptor antagonist triprolidine (0.01 mM) significantly inhibited the initial component of histamine uptake into HUVEC, but did not influence the high capacity transport of histamine. High capacity histamine uptake was significantly increased in the presence of serum containing enzyme diamino-oxidase (DAO), whereas the low capacity uptake of histamine was unaffected in the presence of serum.

Our *in vitro* results suggest that histamine is taken into HUVEC by two independent transport systems. One of them may be OCT3, which is important for the fast component of uptake, but the later phase is carried by another, as yet undefined system, enhanced in the presence of DAO.

P23

Histamine in Beer and "beer allergies".

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Histamine is a product of microbial metabolism especially during fermentation and spoiling of food. In this paper, histamine is determined in beverages like beer before and after mechanical and chemical cleaning of tap installation in selected German bars and restaurants.
Test samples of beer on draught were purchased from 8 typical German bars after mechanical and/or chemical (different antibiotic sterilizations) cleaning of the tap installations. Standardized sensitivity assessments were performed according to the German DIN 10961/ISO 6658 [1]. Biogenic amines were measured fluorimetrically after HPLC, as described previously [1]. After a couple of weeks without cleaning storage devices, tubes and taps, histamine increased up to 35 mg/l histamine in a beer sample on draught. However, this contamination could be significantly reduced after mechanical (35 %) and after combined mechanical/chemical cleaning (93 %), respectively. Besides histamine, other biogenic amines, especially tyramine, were produced and must also be taken into account e.g. in the health risk assessment. Brewing, brewery location and hygienicity were the most important factors for the Enterobacteriaceae, Lactobacillae, Pediococcus and Staphylococcus sps. derived biogenic contamination. Furthermore, after literature scanning, it could be suggested that beer consumption is better than wine, relating to the prevalence of allergic reactions and biogenic amine contamination [2].

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P24

Differential effect of storage on histamine molecular and ultra-molecular dilutions.

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Background : Several papers published during the last 10 years and mainly involving, *in vitro*, histamine and aspirine, were in favour of a biological effect of the so-called high dilutions, i.e. dilutions in which the diluted compound can't be present under a molecular form according to Avogadro's number.
Objectives : As, in our previous works, these dilutions were systematically used during the first weeks after their preparation and stored at 4°C, nothing has been yet been published concerning the effect of storage on their biological activity.
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. The negative feed back of histamine on human basophils was analysed by flow cytometry using the IgE/CD203c protocol and results of the mean fluorescence intensity (MFI) of the two activation markers (IgE and CD203c) were expressed using an index obtained by linearization of the log units given by the cytometer.
Histamine 10^{-4}M was systematically used as a positive control, basophil activation being induced by a target fMLP dilution (10^{-7}M)
Five successive experiments were performed in triplicates, under the same technical conditions and on leukocytes taken from healthy donors immediately after histamine dilutions preparation and after 1, 2 and 3 months of storage at 4°C. Statistical significance was calculated by the Wilkowon distribution free rank test.
Results: fMLP induced a significant IgE downregulation (p=0,015) and a significant CD203c upregulation (P<0,001). Even for the histamine control dilution no effect was observed for the IgE marker whereas the inhibition induced by histamine 10^{-4}M was significant (99%, p<0,001) for the four series of experiments. Percentage inhibition observed for the histamine dilutions 16C and 17C were respectively, for the four series of experiments: 41% (NS), <5%, <5%, <5% and 52% (p=0.02), 63% (p=0.04), 41% (NS) and <5%.
The H_2 receptor-dependant negative feed back induced by histamine on fMLP induced basophil activation was not significant for histamine 10^{-4}M on IgE downregulation but significant on CD203c upregulation in favour of a link between H_2 receptor and non-IgE dependant stimulation. High histamine dilutions were also capable of inducing a negative feedback significant for histamine dilution 17C during the two first months of their preparation. The absence of activity observed after 2 months storage is also in favour of the specificity of the observed effect on freshly prepared dilutions of histamine.

P25

Interest of the measurement of basophil's reactivity and sensitivity for pharmacology and allergy diagnosis.

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Background : *In vitro* basophil activation is usually considered as a yes or no response, activated and resting (not activated) basohils being measured by a global test (mediator release) or by the analysis cell by cell with flow cytometry using selection and activation markers.
Objectives: Using several allergen concentrations, we tested different methods of mathematical approximations in order to check if the activation curve may lead to other information than the global cellular reactivity (measured by the maximum response).
Methods: Basophil activation was analysed by flow cytometry using the IgE/CD63 protocol on leukocytes prepared by buffy coat. Sensitivity and reactivity measurements were tested on hymenoptera venom allergic patients. Three allergen concentrations were systematically tested (by experience 1, 0.3 and 0.1µg/ml of wasp venom purchased from Stallergenes laboratory). Different mathematical approximations were tested but we found that the second degree equation fitted the best the activation curve. Results were then expressed in allergen concentration related to 20% CD63 expression (D20). Three groups of patients were selected, group 1 having experienced a systemic reaction, group 2 having experienced a local or loco-regional reaction and group 3 having been treated by immunotherapy for at least 5 years
Results: Results expressed in %CD63expression alone was not able to differentiate the 3 groups of patients with a clear overlap, whereas the three populations were totally differentiated using the D20 expression with a threshold of 0.3µg/ml between the severe reactions and the local reactions and the patients treated by immunotherapy.
The application of a mathematical treatment to the activation curve led to the calculation of new parameters as the D20 (usual in pharmacology) which combined the maximum response and the slope of the curve leading to a value more discriminating than the simple maximum response.

P26

Synthesis and *in-vivo* evaluation of human histamine H_4 receptor modulators [^{11}C]JNJ7777120 and [^{11}C]VUF10558 for monitoring inflammatory processes using PET.

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The human histamine H_4-receptor (H_4R) is postulated to play a role in a variety of inflammatory conditions such as atopic asthma, allergic rhinitis and rheumatoid arthritis.[1,2] We have developed the radiosynthesis of reference antagonist [^{11}C]JNJ7777120[3] and [^{11}C]VUF10558, a potent H_4R inverse agonist (hH_4R pK_i= 8.35) of undisclosed structure and putative clinical potential. I*n-vivo* evaluation in the rat revealed a very different distribution profile for both radiotracers. These PET-tracers will be used for the investigation of H_4R distribution and monitoring of H_4R expression in inflammatory conditions in animal models.

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P27

Cyclooxygenase Metabolites Reduce Airway Smooth Muscle Response to Histamine in Guinea-pig Trachea.

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Cyclooxygenase (COX) metabolites, such as prostaglandins (PGs) and thromboxan (TX) regulate airway smooth muscle tone during physiological conditions and are suggested to play an important role in asthma. The direct effects of PGE2, PGD2 and TXA2 on the airway smooth muscle are well established but if, and how, they also interfere with responses induced by other agonists, such as histamine, is not clear. Thus, the aim was to study the influence of COX metabolites on histamine contractions, assessed by organ culture of guinea pig trachea rings. Segments were cultured for four days in the absence and presence of the unselective COX-inhibitor indomethacin (INDO; 3 μM). The segments were then placed in tissue-baths with and without INDO supplementation, and their response to increasing concentrations of histamine were assessed (n=6 in each group). When the segments were cultured without INDO and with INDO in the tissue-bath, the contraction response to histamine in the segments was at a similar level to that in fresh segments. However, without INDO during histamine administration, the contraction was approximately 40% lower. This phenomenon was not seen when segments were cultured with INDO. There was no difference in the potency for the histamine based on concentration-response curves. Following administration of acetylcholine or KCl on top of the histamine, contractions were close to the effect of histamine alone. The reason for the reduced contractile response seen in segments cultured without INDO and lacking INDO supplementation is not clear, but a modification of the histamine receptors seems unlikely since the potency remained unchanged. Instead, it is possible that this feature depends on a general mechanism since the overall maximal contraction was reduced. This study indicates that a constitutive secretion of COX metabolites induce an alteration of airway smooth muscle reactivity, decreasing contractile responses to airway constrictors such as histamine, which may have important implications in asthma.

P28

Effects of Oestrogenic Agents on Rat Peritoneal Mast Cells.

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The mast cells have been associated with non-reproduction related actions of estrogens. However, direct effects of oestrogens on mast cells have not been extensively studied. It is, hence, the aim of our study to investigate effects of the natural oestrogen, 17-β oestradiol, the selective oestrogen receptor modulators (SERMs), tamoxifen and raloxifene as well as the specific oestrogen receptor agonists diarylpropionitrile (DPN) and 4',4"-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)- trisphenol (PPT) on rat peritoneal mast cells (RPMC). Histamine release from purified RPMC after various treatments was used an indicator of mast cell activation. The oestrogenic agents alone could initiate low level histamine release (5-10%) from RPMCs. However, suppression of histamine release induced by different secretagogues including anti-IgE, compound 48/80 and substance P was observed when RPMC were activated subsequent to 10 minutes preincubation with the oestrogenic agents. In the case of anti-IgE-induced histamine release, the highest percentages of inhibition by 17-β oestradiol, tamoxifen, raloxifene, PPT and DPN at their corresponding maximally effective concentrations were 44.8 ± 6.3% (10^{-6}M), 42.3% ± 10.8% (10^{-8}M), 69.5% ± 18.1% (10^{-6}M), 42.7% ± 19.6% (10^{-5}M) and 40.2% ± 8.7% (10^{-5}M) respectively. Raloxifene, however, enhanced histamine release between10^{-6} – 10^{-5}M. Similar results were obtained with compound 48/80- and substance P-induced histamine release. In summary, oestrogenic agents produced dual actions on mast cell activation and the rapid development of these actions within 10 minutes suggests the involvement of membrane surface oestrogen receptors.

P29

Increased Levels of Cysteinyl-Leukotrienes in Blood, Urine, Saliva and Induced Sputum from Aspirin-Intolerant Asthmatics.

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There is no simple test to diagnose aspirin-intolerant asthma (AIA). There is, therefore, a great need for new non-invasive methods to measure markers of AIA. It's established that patients with AIA have increased urinary excretion of Cysteinyl-leukotrienes (CysLTs). **AIM:** To compare leukotriene (LT) concentrations in different body fluids (blood *ex vivo*, urine, saliva and sputum supernatant) from patients with AIA and aspirin-tolerant asthma(ATA). **METHODS:** Blood, urine, whole saliva, and induced sputum were collected at baseline from patients with AIA and ATA, as defined by a lysine-aspirin inhalation test at the time of the study. Immunoreactive LTB_4 and E_4 were measured in plasma (from whole blood stimulated *ex vivo* by ionomycin), urine, saliva and the supernatant of processed whole sputum.

RESULTS

Sample	Mediator	Results AIA (ng/mL), n=11, Median (IQR)	Results ATA (ng/mL), n=10, Median (IQR)	p-value
Blood *ex vivo*	LTB_4	**81 (34-109)**	**45 (33-59)**	0.228
Blood *ex vivo*	LTE_4	**55 (36-115)**	**19 (18-32)**	**0.003**
Urine	LTE_4	**79 (66-119) ng/mmol creatinine**	**42 (33-49) ng/mmol creatinine**	**0.001**
Saliva	LTB_4	**0.43 (0.28-0.89)**	**0.39 (0.33-0.57)**	0.460
Saliva	LTE_4	**0.14 (0.11-0.16)**	**0.07 (0.05-0.11)**	**0.007**
Sputum supernatant	LTB_4	**2.6 (1.7-3.6)**	**3.1 (2.3-3.7)**	0.460
Sputum supernatant	LTE_4	**8.2 (7.4-12.3)**	**5.5 (4.1-6.8)**	**0.010**

CONCLUSIONS: The finding of consistently higher levels of CysLTs, but not of LTB_4, in blood *ex vivo*, saliva, and induced sputum are novel. This indicates that increased urinary LTE_4 reflects a global deviation of LT metabolism in AIA.

P30

Effect of histamine on metalloproteinases expression and cell adhesion in breast cancer cell lines.

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Changes in cell adhesion and matrix metalloproteinases production (MMPs) are pivotal for tumour progression to occur. MMPs degrade extracellular matrix, MMP2, MMP9 and MMP7 being associated with the malignant potential of cancer cells. MMPs proteolytic activity is precisely regulated by the endogenous tissue inhibitors of metalloproteinases (TIMPs).
Histamine (HA) has been demonstrated to be an autocrine and paracrine growth factor in several neoplasms, modulating cell survival and invasiveness.
Our aim was to study the effect of HA on cell adhesion, expression and activity of MMP2, MMP7 and MMP9 in human tumorigenic and non-tumorigenic mammary cell lines, MDA-MB-231 and HBL-100, respectively. MMPs protein expression was evaluated by immunocytochemistry, mRNA levels by RT-PCR and gelatinolytic activity by zimography. TIMP1 and TIMP2 mRNA levels were measured by RT-PCR. Cell adhesion was assessed by spectrophotometric measurement of methylene blue stained adherent cells.
HBL-100 showed lower basal gelatinolitic levels than MDA-MB-231 cells. Basal activity and mRNA levels of MMP2 were higher than MMP9 in HBL-100, while MMP9 acitivity and RNA levels were predominant in MDA-MB-231. MMP7 protein and mRNA levels were very low in both cell lines. A significant basal expression of TIMP1 and TIMP2 mRNA levels was observed in these cell lines. 10 microM HA treatment reduced MMP9 and MMP2 activity and mRNA levels in MDA-MB-231 cells, while TIMP1 and TIMP2 mRNA levels were unaffected. In HBL-100, 10 microM HA slightly reduced MMP2 activity. Regarding cell adhesion, it was diminished by 10 microM HA in MDA-MB-231, but increased in HBL100 cells.
These results disclose the ability of HA to modulate MMPs and cell adhesion in both cell lines, suggesting a potential role of HA in the events involved in mammary carcinoma progression.

P31

Blood Histamine Levels in Patients with Inflammatory Bowel Disease on Infliximab Therapy: Preliminary Results.

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Inflammatory cells and mediators like histamine (HIS) and the tumour necrosis factor alpha (TNFα) appear to modulate various components of inflammatory bowel disease (IBD). Infliximab (IFX), a chimeric anti-TNFα monoclonal antibody has been approved for autoimmune diseases and IBD treatment. As evidence relating blood HIS levels and IFX therapy is lacking, this study aimed to assess HIS levels in whole blood of IBD patients maintained in remission on IFX scheduled therapy. We studied 11 patients with Crohn's disease (CD; 8 male, 3 female) or ulcerative colitis (UC; 1 male, 2 female) of median age 35 (range 22-50) and body mass index 23.9 (range 19.3-30). IBD had been diagnosed 1-20 years (median 8) before study entry. Patients were in clinical remission as evidenced by Crohn's Disease Activity Index <150, normal blood C-reactive protein and cell counts. Three healthy volunteers of matching age were included. Patients received IFX 5mg/kg i.v. q8wk for 2-42 months (median 15.5). None developed any acute or delayed allergic reactions. Peripheral whole blood samples were collected before infusion and HIS was extracted and quantified fluorophotometrically. Statistical analyses were performed using SPSS v.13. HIS levels tended to increase in CD patients compared to UC or normal subjects ($p > 0.05$). A stepwise multiple linear regression showed that the gender was the major factor affecting HIS levels in CD patients ($R = 0.877$, $f = 19.9$, beta = 0.877, $p < 0.01$), women showing higher levels ($p < 0.01$) than men or normal subjects. White blood cell (WBC; beta = -0.424, $p < 0.05$) and basophil (beta = -0.286, $p < 0.05$) counts emerged as additional factors. The gender, WBC and basophil counts remained independent predictors of HIS levels for CD and UC patients. A statistically significant correlation ($r = 0.667$, $p < 0.01$) was observed between HIS levels and the number of IFX infusions. These data provide the first evidence linking blood HIS levels to chronic IFX therapy in IBD patients.

P32

A Pilot Study on Peripheral Blood Histamine Levels in Women with Ductal Breast Cancer.

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There is increasing evidence relating histamine (HIS) to cancer. Serum HIS levels in women with breast cancer were reported to be elevated compared to healthy controls [1]. Additional data are required to evaluate the utility of blood HIS as a marker and the significance of any potential HIS-mediated modulating mechanisms in the disease. We aimed at assessing the HIS levels in whole blood obtained from Greek women with breast cancer. In this initial pilot study, we studied 12 women of 45-60 years of age with invasive ductal breast cancer confirmed by histopathological examination of postoperative material (3 *in situ*). Peripheral whole blood samples were collected 1 day either before (n=6) or after (n=2) complication-free surgery performed under endotracheal anaesthesia. Pre- and postoperative blood samples from the same patient (n=5) were also obtained. Patients were on a no medication regimen before surgery and received cephalosporins, analgesic, antiemetic and anticoagulant agents intraoperatively. Age-matched healthy volunteers and women with benign breast lesions (n=5) served as controls. HIS was extracted from blood samples and quantified fluorophotometrically [2]. Statistical analyses were performed using SPSS. Preoperative HIS levels were 86±28 nmol/l whole blood (mean±SD), an order of magnitude higher than those reported for serum [1]. Paired sample statistics revealed significant correlation (r=0.984, p<0.01) between pre- and early post-operative HIS levels in samples of the same patients, with levels being marginally significantly reduced postoperatively. A trend for lower blood HIS levels was noticed in the oestrogen receptor (ER)-negative (n=6) compared to ER-positive (n=3) subgroup, but the difference did not reach statistical significance in this small size sample. The data pointed to the need for studies regarding the role of endogenous HIS among subsets of breast cancer patients.

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P33

Stimulation of H_1 Receptor Inhibits Cell Proliferation through Activation of Small G Proteins in CHO-H_1 Cells.

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The main signaling pathway described for the histamine H_1 receptor (H_1R) includes activation of PLC via Gq. Some GPCRs coupled to Gq/11 activate small Rho GTPases, modulating processes such as proliferation, migration and apoptosis. As histamine (His) modulates cell proliferation through H_1R, the aims of the present study were to find out whether a H_1R and Rho GTPases association existed, to evaluate His effect on CHO-H1 cell proliferation and to determine the link between Rho GTPases and H_1R-induced proliferation.
The studies were performed in CHO cells stably transfected with H_1R. Pull down assays precipitating the GTP-bound forms of the main Rho GTPases, showed that His and H_1 agonists induced Rac1 and RhoA activation in a time and dose-dependent manner, but not of Cdc42. The response was abolished by mepyramine (H_1R antagonist) and by U73122 (PLC inhibitor) supporting that Rac 1 and RhoA activation was mediated by H_1R coupled to PLC stimulation. Rho modulation in cells can be monitored by serum response factor activity with the SRE-luciferase reporter system. Stimulation of CHO-H1 with His led to a 3-fold increase in luciferase activity. The response was inhibited by the coexpression with C3 toxin, which inactivates Rho, strongly supporting that His stimulates downstream signals through RhoA activation. In addition, Rac-dependent JNK activation was detected by western blot within 30 min of His stimulation, inhibited by β2-chimaerin (Rac-GAP). The stimulation of H_1R also led to ERK1/2 activation which was not dependent on either Rho or Rac. [^{3}H]-thymidine incorporation assays showed that His and the H_1 agonist inhibited cell proliferation in a dose-dependent fashion. Inhibition of RhoA and Rac by expressing either C3 toxin or β2-chimaerin, respectively revealed that Rac was partly involved in His inhibitory effect.
Present findings support that Rho GTPases activation constitutes a new step in the H_1R signaling pathway that leads to the inhibition of cell proliferation.

P34

The effect of iodinated and gadolinium contrast media on the histamine degrading enzymes diamine oxidase and histamine methyltransferase – an in-vitro study.

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Objectives:
The biochemical pathways of severe contrast medium associated adverse reactions including nephorgenic systemic fibrosis (NSF) and anaphylactoid reactions are not completely understood. In man, histamine can be catabolised either by diamine oxidase (DAO) or by histamine methyltransferase (HMT). Numerous drugs have been reported to inhibit histamine metabolism. Since histamine has been attributed a stimulating effect in fibrogenesis and is known as an important mediator in allergy, it was the aim of this study to investigate, if gadolinium-containing or iodinated contrast agents have an inhibitory effect on activities of DAO and HMT.
Methods:
Seven gadolinium-based (Gadoterate meglumine, Gadoteridol, Gadobutrol, Gadobenate dimeglumine, Gadopentetate dimeglumine, Gadoxetate disodium, Gadodiamide), and eight iodinated contrast media (Iodixanol, Iotrolan, Ioversol, Iobitridol, Iopamidol, Iomeprol, Iothalmate meglumine, Amidotrizoic acid) were tested *in vitro*. Contrast agents were incubated at various concentrations (0.1-10 mM) with purified DAO and recombinant HMT, respectively, and enzyme activities were determined using radiometric micro assays. As controls, enzyme activities were measured without addition of contrast agents and after incubation with specific inhibitors of DAO or HMT.
Results:
The tested gadolinium-based and iodinated contrast media did not relevantly inhibit enzyme activities of DAO and HMT, whereas specific inhibitors as positive controls showed an inhibitory effect. No significant difference was observed between ionic and non-ionic substances, between cyclic and linear gadolinium chelates or between monomeric and dimeric iodinated contrast agents.
In conclusion, gadolinium-based and iodinated contrast media show *in vitro* at physiological, concentrations, no inhibitory effect on histamine metabolism.

P35

Histamine chloramine modifies casein-induced inflammation.

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We have previously shown *in vitro* that histamine chloramine (HisCl), an endogenous compound formed at the inflammatory sites from HClO (activated neutrophils) and histamine (mast cells), inhibited reactive oxygen production in neutrophils via the H4 receptor. In this study, we evaluated the effect of HisCl on development of acute inflammation: casein- induced inflammation in rats.
Inflammation was induced by injection of 12% solution of casein into peritoneal cavity of Wistar rats. Rats were treated intraperitoneally with HisCl (5ml 5Mm i.p.) twice, 2 hours prior and 4 hours after casein administration. Thioperamide maleate (2mg/kg) - histamine H3/H4 inhibitor and ciproxifan hydrogenmaleate (0.14 mg/kg) – histamine selective H3 inhibitor, were administered i.p. 1 hour before HisCl.

Administration of HisCl prior to casein and during development of acute inflammation suppressed the generation of oxygen radicals in peripheral blood neutrophils. Thioperamide blocked the inhibitory effect of HisCl on the oxidative burst of neutrophils. Ciproxifan was unable to antagonize the suppressing effect of HisCl.
In the course of casein-induced inflammation, elevated level of histamine in blood was found. HisCl caused an additional increase in histamine level.
This preliminary study shows the effects of *in vivo* HisCl administration on the development of acute inflammation, indicating that exogenous HisCl may modulate the course of experimental inflammation through a mechanism that partly involves stimulation of production/release of histamine and production of oxygen free radical species by blood neutrophils.

P36

Increased expression of histamine H_1 receptor by human amniotic epithelial cells (HAEC) in chorioamnionitis (CHA) is accompanied by augmented production of secretory leukocyte protease inhibitor (SLPI).

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Increased epithelial expression of histamine H_1 receptor has been reported in inflammatory conditions, including CHA. HAEC produce SLPI – an effective regulator of important immunological functions. Anti-inflammatory and anti-microbial activities of SLPI within fetal membranes may modulate the course of CHA. In this ***in vitro*** study, we examined comparatively (histological CHA-complicated vs normal pregnancy; Group I and II, respectively) the correlation between H_1 receptor expression and SLPI level within cultured HAEC.
HAEC were isolated from the amnion after term pregnancies and cultured in normoxia in 24-well culture plate inserts (1.0 million cells per well) in Ham's F12 and Dulbeco's modified Eagle medium supplemented with 10% fetal calf serum. Twelve placentas have been obtained and 72 cultures were established within each group. The human SLPI ELISA was used for the quantitative measurement of SLPI in HAEC culture medium on day 5 and after challenge with histamine (100μM) on day 8. In order to examine the effect of H_1 blockade, pyrilamine (20nM) was added in respective subgroups. After 5 or 8 days – depending on the subgroup – the supernatants were removed and the cultures formalin-fixed and paraffin-embedded for H_1 receptor immunostaining. Quantitative immunohistochemistry was applied for evaluation of H_1 expression.
Mean concentration of SLPI was significantly increased ($p<0.05$) in Group I compared to Group II, both on the 5th (6.61 ±1.83 vs 3.34 ±1.41; μg/ml ±SEM) and the 8th day (23.03 ±4.78 vs 10.17 ±2.37). Histamine stimulated SLPI production and this effect was almost cancelled by histamine H_1 blocker. Mean expression of H_1 was significantly increased ($p<0.01$) in CHA amounting to 173.06 ±15.98 (%, ±SEM) of the reference value (Group II).
Considering that SLPI can inhibit IgE-mediated histamine release from mast cells, our present results indicate, that SLPI may be involved in another compensatory mechanism, acting as an anti-histaminergic agent.

P37

Increased permeability of human amnion to calcium ions in chorioamnionitis (CHA) is related to histamine H_1 receptor overexpression within amniotic epithelial cells (AEC).

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Changed fetal membranes may facilitate inflow of Ca++ ions, increasing myometrial contractile activity. Histamine increases permeability of biological membranes acting via its H_1 receptor, especially during inflammation. We examined comparatively (CHA-complicated vs normal pregnancy) the relationship between permeability of human amnion to Ca++ and histamine H_1 receptor expression in corresponding AEC.
Pieces of isolated extraplacental amnion with histological CHA obtained after "near to term" labours (N=16) were compared with gestationally matched normal amnion samples (Group I and II, respectively). Diffusion of Ca++ was measured in a system of plastic container divided into two chambers by the mounted amnion and filled with 0.9% saline solution. The effects of histamine (concentration range: 10-500μM) and H_1 receptor blockade (pyrilamine, 0.1μM) on the transport coefficient for Ca++ were examined in respective subgroups. Moreover, at the time of amnion sample collection, AEC have been isolated from each amnion subjected to permeability assay. These cells were then cultured in 24-well culture plate inserts in Ham's F12 and Dulbeco's modified Eagle medium with 10% fetal calf serum. After 5 days, the supernatants were removed and the cells formalin-fixed and paraffin-embedded for H_1 receptors immunostaining. Quantitative immunohistochemistry was applied for evaluation of H_1 expression.
Both, basal and histamine-related permeability for Ca++ were significantly increased in CHA. Pyrilamine diminished the effect of histamine on amnion permeability to Ca++ proportionally in both groups (the mean permeability decrease %,± SEM: 45,65±13.07 to 70±12.28, depending on histamine concentration). Mean expression of H1 was increased ($p<0.02$) in CHA (the cell cultures developed from Group I) and amounted to162.41±16.03 (%,±SEM) of the reference value (Group II).
In conclusion, overexpression of H_1 in amniotic epithelium during CHA may cause preterm, calcium-induced uterine contractions.

P38

Histamine Inhibits Proliferation through the Four Histamine Receptor Subtypes in MCF-7 Human Breast Cancer Cell Line.

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We have reported that histamine (HA) regulates differentially signaling processes in HBL-100 normal and MDA-MB-231 malignant [estrogen receptor (ER) α-] human breast cells. The aim of the present study was to investigate the biological responses triggered by HA in MCF-7 (ERα+) breast cancer cells. For this purpose we determined the expression of the HA receptors (HR) subtypes, H_1R, H_2R, H_3R and H_4R by RT-PCR; histidine decarboxylase (HDC) by western blot; HA content by immunostaining; cAMP production by RIA; cell proliferation by the clonogenic assay; differentiation by Nile red staining and flow cytometry; and apoptosis by flow cytometry and TUNEL assay. Results indicate that MCF-7 cells expressed the four HA receptors, HDC and presented a moderate level of intracellular HA. HA treatment (10 μM) produced a 2-fold increase in cAMP levels and reduced cell proliferation (30%). Lower HA doses also decreased proliferation. By using specific HA agonist and antagonist, we determined that HA decreased proliferation through the stimulation of the four HA receptors subtypes however, the most significant effect was exerted via H_4R (70%). HA was unable to promote differentiation in these cells. On the other hand, the inhibitory effect of HA on proliferation was associated with an induction of apoptosis after 72 h of treatment. Present results show that HA was incapable of inducing proliferation via the H_3R in these cells as compared with the more undifferentiated MDA-MB-231 breast cancer, suggesting that different subtype expression, protein-protein interactions, or localization could be responsible for the differences in the signaling pathways.

P39

The Activity Of Histamine Degrading Enzymes And Uptake Of Histamine In Human Vascular Endothelial Cells: Influence Of Antidepressants.

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Specific stimuli cause the release of histamine from histamine-containing cells. The release of histamine and its effects in the body have been profoundly studied, but its disappearance from the circulation and the extracellular space is still not entirely understood. Several pathways of histamine uptake have been suggested, including vesicular monoamine transporter 2, organic cation transporter, and histamine degradative uptake. In the present study, we investigated the activity of histamine degrading enzymes, diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT) in cultured human umbilical cord endothelial cells, and the indication for one of the uptake pathways, the histamine degradative uptake. Since our previous studies have shown that antidepressants can modulate the pharmacokinetics of histamine, we examined also effects of amitriptyline and sertraline. Thus we studied changes in [^{3}H]-histamine uptake upon action of the DAO inhibitor (aminoguanidine), HNMT inhibitor (amodiaquine), and the antidepressants, amitriptyline and sertraline. DAO and HNMT activity in endothelial cells was determined after no treatment and after treatment with aminoguanidine, amitriptyline and sertraline. Aminoguanidine lowered the [^{3}H]-histamine uptake by 20%, while amodiaquine did not change its rate. Sertraline lowered [^{3}H]-histamine uptake in a concentration-dependent manner and amitriptyline increased [^{3}H]-histamine uptake into endothelial cells. DAO activity in endothelial cells was demonstrated, although its activity was low; however, we could not detect any HNMT activity. Aminoguanidine and sertraline lowered DAO activity, whereas amitriptyline increased DAO activity. The results indicate that in human cultured endothelial cells, the histamine degradative uptake contributes to the process of histamine uptake. This study also shows that antidepressants influence histamine uptake.

P40

Histamine H3 Receptor Antagonists: Correlation of Pharmacokinetic and Pharmacodynamic Effects with Physical Properties

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Advances in molecular biology have afforded medicinal chemists with a plethora of new targets for investigation. The cloning of the histamine H3 receptor [1] is one of many recent examples where target discovery was rapidly followed by high throughput screening to find starting points for medicinal chemists. However the transformation of typically weak, non-selective and non-drug like leads into therapeutic agents often remains a challenge. We have previously described several series of potent and selective histamine H3 antagonists [2] and noted the consequences of structural changes on pharmacokinetic behavior [3]. This prompted a more detailed examination of physical properties and their correlation with drugability. The impact of property data on pharmacokinetics and pharmacodynamics for two series of structures will be presented.

[1] Lovenberg, T. W., Roland, B. L., Wilson, S. J., Jiang, X., Pyati, J., Huvar, A., Jackson, M. R., Erlander, M. G. Mol. Pharmacol. 1999, 55, 1101.
[2] Bonaventure, P.; Letavic, M.; Dugovic, C.; Wilson, S.; Alusio, L.; Pudiak, C.; Lord, B.; Mazur, C.; Kamme, F.; Nishino, S.; Carruthers, N.; Lovenberg, T. "Histamine H3 Receptor Antagonists: From Target Identification to Drug Leads", Biochemical Pharmacology, (2007), 73(8), 1113-1122.
[3] 33rd Meeting of the European Histamine Research Society, Cologne, Germany, April 28th-May 2nd 20044

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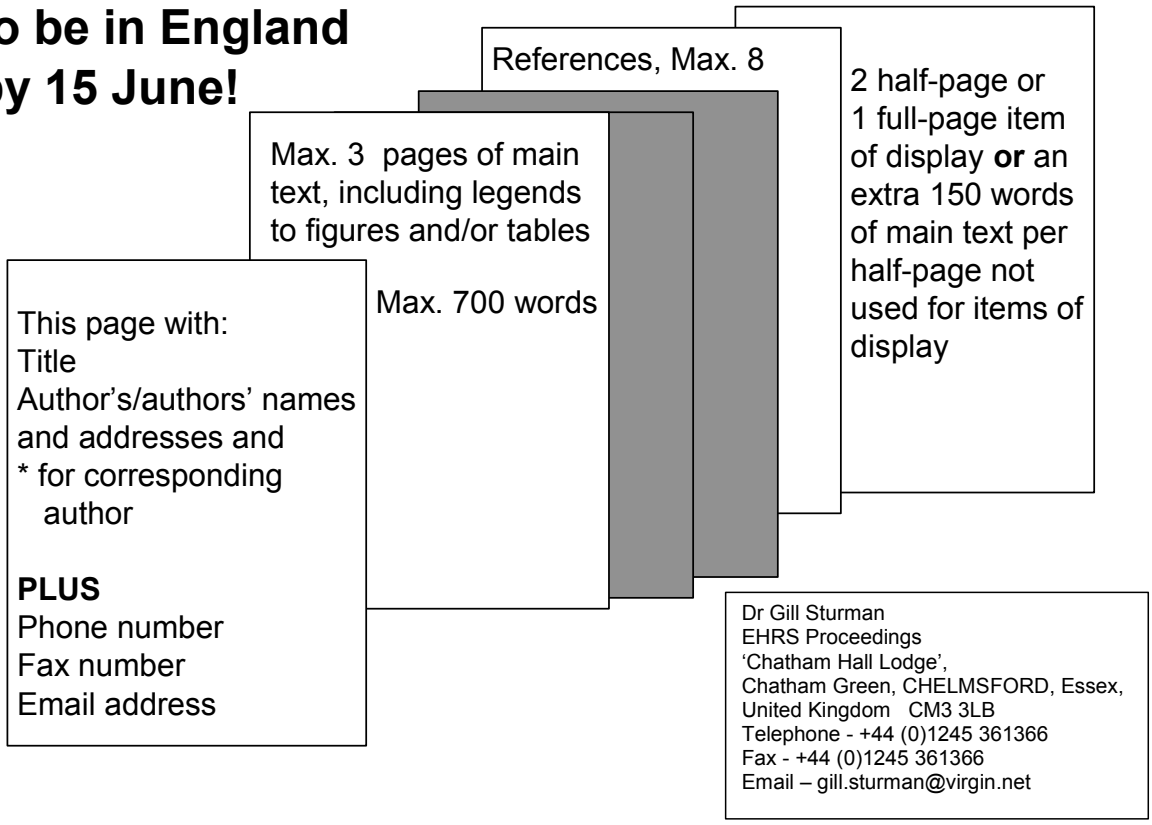

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Siegel S, Nonparametric Statistics for the Behavioral Sciences. Tokyo: McGraw-Hill-Kogakusha, 1956:116-27.

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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.

CHORUS

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