EUROPEAN HISTAMINE RESEARCH SOCIETY

PROGRAMME AND ABSTRACTS

DELPHI GREECE

10 - 13 may 2006

eHRS

XXXV MEETING

Under the auspices of the Medical School of the University of Athens & the Prefecture of Fokida

Medical School of the University of Athens
DEPARTMENT OF PHARMACOLOGY
http://conferences.med.uoa.gr/ehrs-06

CONTENTS

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Poster Design: Dimitrios Kourkoutis

Previous EHRS Annual Meetings

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

WELCOME

10 May 2006

Myth and science meet at Delphi, the navel (Gk: omphalos) of the earth that owes its reputation to the oracle of Apollo, where the god's divination was given through the mouth of his prophetess Pythia.

On behalf of the Organising Committee, I welcome you to the XXXVth Meeting of the European Histamine Research Society at the European Cultural Centre of Delphi. This annual EHRS Meeting is organised, for the first time in Greece, by the Department of Pharmacology of the Medical School of the University of Athens.

The Organizers and the Scientific Committee made every effort to ensure that all presentations are filled with the scents and the sounds of the most motivating up-to-date scientific information bound together in a radiant circle of histamine research. The sessions will focus on the chemistry and pharmacology of the four histamine receptor subtypes, on established and novel functions of the amine in inflammation, cognition, metabolism, reproduction, differentiation, immunology, neuro-immuno-endocrinology, cancer and genomics. Three stimulating invited talks will surround these topics. The president of the EHRS, Prof M. Ennis will guide the audience through the achievements in histamine research in the 35 year long history of the Society; Prof A. Falus will introduce the future perspectives of histamine in systems biology; Prof P. Maestrelli will portray the clinical dimension in the "D Varonos Memorial Symposium". The continued interest in histamine research will be revealed in the competitive "Art Hancock Young Investigator Award Symposium". Finally, we are delighted that Ingrid Uvnäs will be presented with an Honorary Membership, for her support to our Society over the years.

Thanks are due to the participants who made this event happen. Travelling across paths paved with unlimited affection for research, they came from their homelands to this corner of the Mediterranean to disclose effective roles of histamine in an effort to ameliorate the art of life. This journey would be impossible without the valuable assistance of our PhD students and the kind support of the sponsors, who contributed to the materialization of this histamine feast around the oracle of Delphi, under the spirit of wondering principles of our ancestors.

Delphi is a place of inexhaustible mythological and historic references. The motivating scientific sessions together with the archaeological site of Delphi, the picturesque varied scenery of Mount Parnassos, the olive tree valley, the Corinthian Gulf harbours of Galaxidi, Kirra and Itea and the contemporary cultural life and natural beauty of the region hopefully will provide infinite opportunities for a most pleasant stay.

Welcome to Delphi!

On behalf of the organising committee

Catherine Tiligada

Location of the Meeting
European Cultural Centre of Delphi (ECCD), Greece

Host Institute
Department of Experimental Pharmacology
Medical School, University of Athens, M. Asias 75, GR-11527 Athens, Greece

President of the EHRS
Professor Madeleine Ennis
The Queen's University of Belfast, UK

Chairperson of the Meeting
Dr Ekaterini Tiligada
University of Athens Medical School, Greece

Organising Committee
Andreas Delitheos
Madeleine Ennis
Vassiliki Giannoulaki
Sotirios Kakavas
Zoe Papadopoulou-Daifoti
Christina Spyraki
Ekaterini Tiligada

Executive Assistants
Vassilios Delitheos
Konstantinos Papamichael
Evangelia Zampeli

Abstract Evaluation & Bursary Selection Committee
Madeleine Ennis (Chairperson)
Patrizio Blandina
Andras Falus
Tatjana Irman Florjanc
Pertti Panula
Gill Sturman
Anita Sydbom

The Art Hancock Young Investigator Award Jury
Walter Schunack (Chairman), Timothy A Esbenshade, Pertti Panula

Poster Jury
Frank Ahrens (Chairman), El-Sayed K Assem, Patrizio Blandina, Bernhard F Gibbs, Nina Grosman, Mariusz Gujski, Krisztina Hegyi, Zsuzsanna Huszti, Tatjana Irman-Florjanc, Emanuela Masini, Danuta Maślińska, Elena S Rivera, Elke Schneider, Holger Stark, Gill Sturman, Anita Sydbom

GENERAL INFORMATION

Registration Desk

The registration desk will be open:

Wednesday	May 10th	15:00 – 21:00	at the Delphi Palace Hotel
Thursday	May 11th	08.00 – 13.00	at the ECCD
Friday	May 12th	08.00 – 18.00	at the ECCD
Saturday	May 13th	08.00 – 13.00	at the ECCD

Congress Website and E-Mail / Internet Service

http://conferences.med.uoa.gr/ehrs-06

E-mail and Internet services is provided at the Delphi Palace Hotel

Oral Communications

They should last no longer than 10 min (plus 5 min for discussion), exept for the EUBREN Basophil Network Symposium, which is scheduled for 15 min (plus 5 min discussion). A PC equipped with MS PowerPoint 2003 for Data Projection is available. The presentations should be brought in a USB memory stick (MS PowerPoint format). Presentation preview: On Wednesday May 10th, 15.00-21.00, at the Registration desk in Delphi Palace Hotel and the following days during the first coffee break, in the ECCD Conference Hall.

Poster Presentations

The posters will be on display throughout the meeting. Presenters should present the main point(s) from the poster in 1 min (followed by 4 min discussion) in the corresponding session. They are encouraged to stand by their posters during the coffee breaks. Short-listing posters for the poster prizes (announced on Saturday 13th, 14.00) will be revisited by the Poster Jury on Saturday 13th, 16.30-17.00. Prizes will be announced at the Farewell Dinner.

Accommodation Information

Guest House "EUROPA"

Delphi Palace	69 Apollonos Str, 33054 Delphi	Tel: +30 2265082151
Hotel King Iniohos	78 Osiou Louka Str, 33054 Delphi	Tel: +30 2265082701
Hotel Iniohos	19 Vas. Pavlou & Friderikis, 33054 Delphi	Tel: +30 2265082710

Social Events

Wednesday	May 10th	19:30	Welcome reception on the terrace of "Delphi Palace Hotel" (dress: informal)
Thursday	May 11th	15:00	Visit to the Delphi archaeological site and museum
		17:30	Visit to the Monastery of "Profitis Elias"
		19:00	Visit and dinner in the harbor of Galaxidi. (dress: mid-season clothes, comfortable shoes, light sweatshirt/raincoat)
Friday	May 12th	20:00	Dinner at the Piano Restaurant "Iniohos" (dress: informal)
Saturday	May 13th	20:00	Farewell Dinner at the "Villa Symposium" (dress: lounge suit/elegant dress recommended but not essential)

Transfer to Athens International Airport "El. Venizelos" on Sunday 14th

See announcement at the registration desk.

THE PROGRAMME AT A GLANCE

Wednesday 10 May 2006	
15.00 - 21.00	Arrival - Check in - Registration
18.00 - 19.30	EHRS Council Meeting
19.30 - 22.30	Welcome reception
Thursday 11 May 2006	
08.00 - 13.00	Registration
08.00 - 08.45	Opening ceremony
08.45 - 09.30	**Invited lecture**: "Histamine and the EHRS – what has happened in the last 35 years?" Madeleine Ennis
09.30 - 10.30	**Oral session 1**: Future perspectives in histamine research
10.30 - 11.45	Coffee break
10.45 - 11.45	**Poster session 1**: H_3 receptors
	Poster session 2: Mast cells and Basophils
11.45 - 13.00	**Oral session 2**: Allergy and Inflammation
13.00 - 13.30	**Poster session 3**: Histamine in the CNS
	Poster session 4: Histamine in the GI tract
13.30 - 14.30	Lunch
15.00 - 22.00	Excursion - Dinner
Friday 12 May 2006	
08.00 - 19.00	Registration Desk Open
08.00 - 08.45	**GB WEST lecture**: "Histamine and systems biology; genes and genomics beyond genes" Andras Falus
08.45 - 09.45	**Oral session 3**: Metabolism, Reproduction and Cancer
09.45 - 11.15	**Oral session 4**: Histamine and brain
11.15 - 12.00	Coffee break
11.30 - 12.00	**Poster session 5**: Clinical aspects of histamine research
	Poster session 6: Histamine in Reproduction and Cancer
12.00 - 13.30	**The Art Hancock Young Investigator Award Symposium**
13.30 - 14.30	Lunch
14.30 - 17.00	**The Art Hancock Memorial Symposium**
17.00 - 18.00	Coffee break
17.15 - 17.50	**Poster session 7**: Immunology
	Poster session 8: Receptors and other subjects
20.15 - 22.00	Dinner
Saturday 13 May 2006	
08.30 - 13.00	Registration Desk Open
08.30 - 09.15	**Invited lecture**: "Occupational asthma: diagnosis and management" Piero Maestrelli
09.15 - 10.30	**The D Varonos Memorial Symposium**: "Clinical implications – histamine and disease"
10.30 - 11.00	Coffee break
11.00 - 12.50	**Oral session 5**: Neuro-immuno-endocrine aspects of histamine
13.00 - 14.00	Lunch
14.00 - 16.30	**The EUBREN Basophil network symposium**
16.30 - 17.00	Coffee break
16.30 - 17.00	Poster evaluation Committee
17.15 - 19.00	General assembly
20.30	Farewell Dinner
Sunday 14 May 2006	
	Check out - Departure

SCIENTIFIC PROGRAMME

The meeting starts in the afternoon of Wednesday 10 May 2006 and closes officially after the farewell dinner in the early hours of Sunday 14 May 2006.

Wednesday 10 May 2006

15.00-21.00		Arrival - Check in - Registration
18.00-19.30		EHRS Council Meeting
19.30-22.30		Welcome reception

Thursday 11 May 2006

08.00-13.00		Registration
08.00-08.45		**Opening ceremony**
08.45-09.30	I1:	**Invited lecture** Histamine and the EHRS – what has happened in the last 35 years? Madeleine Ennis, UK Introduced by *Prof Fred L Pearce*
09.30-10.30		**Oral session 1: Future perspectives in histamine research** Chairpersons: *Prof Fred Pearce & Prof Agnieszka Fogel*
09.30-09.45	O1:	Novel ligands stabilize stereo-selective conformations of the H_1 receptor to result in functionally-selective activation or antagonism of intracellular signaling pathways *Booth RG*
09.45-10.00	O2:	Fast production and analysis of the human histamine H_1 receptors using a cell free protein expression system followed by MS analysis *Sansuk K, Bakker RA, Hensbergen P, Leurs R*
10.00-10.15	O3:	Effect of sulphasalazine and balsalazide on histamine release from mast cells *Peh KH, Wan BCY, Assem ESK, Pearce FL*
10.15-10.30	O4:	Luminometric determination of amine oxidase activity *Schwelberger HG, Feurle J*
10.30-11.45		Coffee break
10.45-11.45		**Poster session 1: H_3 receptors** Chairpersons: *Prof Patrizio Blandina & Prof Holger Stark*
	P1:	H_3 receptor activation phosphorylates Akt in rat cortical neurons *Mariottini C, Chiarugi A, Fossati S, Blandina P, Passani MB*
	P2:	Histamine H_3 receptors modulate [^{3}H]-dopamine release in rat *substantia nigra pars reticulata*, but not in prefrontal cortex *Ramos-Jiménez J, Garduño-Torres B, Camacho J, Arias-Montaño J-A*
	P3:	Is the high affinity selective H_3 receptor agonist methimepip anxiolytic or anxiogenic in a new validated animal models of human anxiety? *Chazot PL, Leurs R, de Esch IJP, Ennaceur A*

P4: Down-regulation of histamine H_3 receptors in rat striatum
Garduño-Torres B, Arias-Montaño J-A

P5: Pharmacological characterization and *in vitro* ADME properties of immethridine, a potent and highly selective histamine H_3-R agonist
Celanire S, Gillard M, Christophe B, Quere L, Collart P, Dassesse D, van Marle A, Hulscher S, Bakker R, Zuiderveld P, Timmerman H, Leurs R, Chatelain P, Lamberty Y, Talaga P

P6: Histamine H_3 receptors regulate glutamate, but not GABA release in rat thalamus
Garduño-Torres B, Treviño M, Gutiérrez R, Arias-Montaño J-A

P7: Heterocyclic replacements for the central phenyl-core of diamine–based human histamine H_3 receptor antagonists
Carruthers NI

P8: Histamine 3 receptor inverse agonists for the treatment of obesity. Biological and chemical challenges
Freichel C, Gatti-Mac Arthur S, Hertel C, Huwyler J, Nakagawa T, Nettekoven M, Plancher J-M, Raab S, Richter H, Roche O, Sarmiento RMR, Schuler F, Taylor S, Ullmer C, Wiegand R

P9: Ureas with H_3-antagonist activity–a new scaffold discovered by lead-hopping starting from cinnamic acid amides
Lau J, Jensen CB, Lund P, Hohlweg R

P10: Radiosynthesis and biodistribution of a histamine H_3 receptor antagonist: evaluation of a potential pet ligand
Airaksinen AJ, Jablonowski JA, Van der May M, Barbier AJ, *Klok RP, Verbeek J, Schuit R, Herscheid JDM, Leysen JE, Carruthers NI, Lammertsma AA, Windhorst AD*

P11: (3-Piperidin-1-yl-propoxy)-tetrahydroisoquinolines and tetra-hydroazepines: A novel series of selective histamine H_3 receptor antagonists
Jesudason CD, Beavers LS, Cramer JW, Dill J, Finley DR, Gleason SD, Hemrick-Luecke SK, Lindsley CW, Nelson DLG, Stevens FC, Gadski RA, Oldham SW, Pickard RT, Siedem CS, Sindelar DK, Singh A, Watson BM, Witkin JM, Hipskind PA

10.45-11.45 **Poster session 2: Mast cells and Basophils**
Chairpersons: *Dr Nina Grosman & Dr El-Sayed K Assem*

P12: Mast cell-derived interleukin-8 may be involved in the ovarian mechanisms of follicle growth and ovulation
Szukiewicz D, Pyzlak M, Klimkiewicz J, Szewczyk G, Maślińska D

P13: Potent histamine-releasing activity of atrahagin, a snake venom metalloproteinase
Wei JF, Mo YZ, Qiao LY, Wei XL, Chen HQ, Xie H, Fu YL, He SH

P14: Gene expression profiling of mouse mucosal mast cell (mMMC) proteases
Wiener Z, Keszei M, Gilicze A, Falus A

P15: Distribution of mast cell- tryptase and metallothionein in human brains with amyloid deposit
Maślińska D, Laure-Kamionowska M, Maśliński KT, Gujski M, Maśliński S

P16: Clinico-biological characteristics of flow cytometry applied to NSAIDs hypersensitivity diagnosis. Determination of preliminary ROC curves
Sainte-Laudy J, Touraine F, Boumediene A, Cogné M

P17: Amitriptyline inhibits mast cell histamine secretion: implications for chronic fatigue syndrome therapy
Theoharides TC, Kempuraj D

P18: Lack of endogenous histamine affects early inflammatory phase of wound healing
Holub MC, Falus A

P19: Transtympanic *versus* intramuscular steroid administration in a histamine-induced inflammatory middle ear model
Chimona TS, Panayiotides JG, Papadakis CE, Helidonis ES, Velegrakis GA

P20: A heretofore undisclosed crux of eosinophilia-myalgia syndrome: compromised histamine degradation
Smith MJ, Garrett RH

P21: Music therapy, "adverse" diet and histamine
Hanke A, Klawitter B, Meng H, Herwald M, Hasenmaier M, Borck H, Fischer M, Diel E, Diel F

P22: Analysis of human basophil activation feed back induced by histamine and high histamine dilutions
Sainte-Laudy J, Belon Ph

P23: *Whithdrawn*

11.45-13.00 Oral session 2: Allergy and Inflammation
Chairpersons: *Prof Friedhelm Diel & Prof Marija Čarman-Kržan*

11.45-12.00 O5: Activation of cannabinoid receptors prevents allergen-induced asthma-like reaction in sensitized guinea pigs
Fabrizi F, Vannacci A, Giannini L, Mariottini C, Passani MB, Mannaioni PF, Nistri S, Masini E

12.00-12.15 O6: Effects of the carbon monoxide releasing molecule CORM-3 in a coincubation model with rat mast cells and human neutrophils
Vannacci A, Giannini L, Fabrizi F, Uliva C, Mastroianni R, Masini E, Mannaioni PF

12.15-12.30 O7: Prevention of antigen-induced bronchoconstriction by epigallocatechine-3-gallate in actively sensitized guinea pigs
Giannini L, Uliva C, Bani D, Mastroianni R, Suzuki Y, Nistri S, Mannaioni PF, Suzuki H, Masini E

12.30-12.45 O8: Increased nitric oxide production mediates T cell signal transduction in histidine decarboxylase knockout mice
Koncz A, Nagy G, Mazan M, Buzás E, Falus A

12.45-13.00 O9: Histamine influences the DNA interaction of STATs in human lymphocytes
Diel F, Borck H, Meng H

13.00-13.30 **Poster session 3: Histamine in the CNS**
Chairpersons: *Dr Zsuzsanna Huszti & Dr Gill Sturman*

P24: *Whithdrawn*

P25: Excitatory effect of histamine on neuronal activity of rat globus pallidus by activation of H_2 receptors *in vitro*
Chen K, Wang J-J

P26: *Whithdrawn*

P27: Histamine improves rat rota-rod and balance beam performances through H_2 receptors in the cerebellar interpositus nucleus
Song Y-N, Li H-Z, Zhu J-N, Wang J-J

P28: P2Y receptor mediated excitation in the posterior hypothalamus
Sergeeva OA, Klyuch BP, Fleischer W, Eriksson K, Korotkova TM, Siebler M, Haas HL

P29: Central histaminergic system – the regulatory mechanism of circulatory homeostasis in haemorrhagic shock
Jochem J

13.00-13.30 **Poster session 4: Histamine in the GI tract**
Chairpersons: *Dr Frank Ahrens & Dr Hegyi Krisztina*

P30 Amitriptyline affects guinea pig post-heparin plasma diamine oxidase activity
Rajtar S, Irman-Florjanc T

P31: Immunolocalization of histamine H_3 receptor in the rat gastrointestinal tract
Chazot PL, Shenton FC, Schunack W, Grandi D, Morini G

P32: Influence of H_3/H_4 receptor antagonist thioperamide on regional haemodynamics in rats with trinitrobenzene sulfonic acid-induced colitis
Fogel AW, Jochem J, Lewinski A

P33: *Whithdrawn*

P34: Functional gut mucosal biopsy testing using tolerated food antigens during mucosa oxygenation yields a high extent of specificity in non-atopic, non-allergic healthy individuals
Raithel M, Sehnert C, Nägel A, Backhaus B, Straube S, Buchwald W, Schultis W, Kressel J, Hahn EG, Kimpel S, Konturek P

P35: Mechanisms underlying the radioprotective effect of histamine on small intestine
Medina VA, Mohamad NA, Croci M, Cricco GP, Núñez MA, Martín GA, Bergoc RM, Rivera ES

13.30-14.30	Lunch
15.00-22.00	Excursion - Dinner

Friday 12 May 2006

08.00-19.00 Registration Desk Open

08.00-08.45 I2: **GB WEST lecture**
Histamine and systems biology; genes and genomics beyond genes
Andras Falus, Hungary
Introduced by *Prof Madeleine Ennis*

08.45-09.45 **Oral session 3: Metabolism, Reproduction and Cancer**
Chairpersons: *Prof Madeleine Ennis & Dr Hubert Schwelberger*

08.45-09.00 O10: Amine system project
Medina MA, Aldana JF, Villatoro FR, Claros G, Urdiales JL, Trelles O, Sánchez-Jiménez F

09.00-09.15 O11: Effects of histamine on triglyceride breakdown in mouse adipocytes
Carpéné C, Bour S, Prévot D, Duffaut C, Valet P

09.15-09.30 O12: Highlights in histamine function and reproduction
Pap E, Pállinger E, Falus A

09.30-09.45 O13: Expression of histamine H_3 and H_4 receptors in benign lesions and malignant carcinomas of the human mammary gland
Medina VA, Croci M, Crescenti EJV, Bergoc RM, Rivera ES

09.45-11.15 **Oral session 4: Histamine and brain**
Chairpersons: *Dr Paul Chazot & Dr Zoe Papadopoulou-Daifoti*

09.45-10.00 O14: Generation of the first anti-hH_4 receptor antibody: identification of the H_4R in human lymphocytes and brain
Chazot PL, Shenton FC, Van Rijn RM, Bakker RA, Leurs R

10.00-10.15 O15: Aversive memory consolidation and reconsolidation: differences in neurotransmitter engagement.
Bucherelli C, Baldi E, Mariottini C, Passani MB, Blandina P

10.15-10.30 O16: Properties of native $GABA_A$ receptors in histaminergic and orexinergic neurons
Sergeeva OA, Haas HL

10.30-10.45 O17: Characteristics of cortical EEG and sleep-wake cycle in *histamine H_1-receptor knockout mice*
Parmentier R, Anaclet C, Watanabe T, Lin J-S

10.45-11.00 O18: Histamine and behavioral state-dependent control of hippocampal plasticity
Selbach O, Haas HL

11.00-11.15 O19: *Whithdrawn*

11.15 -12.00 Coffee break

11.30-12.00 **Poster session 5: Clinical aspects of histamine research**
Chairpersons: *Dr Anita Sydbom & Dr Mariusz Gujski*

P36: *Whithdrawn*

P37: Evaluation of urinary N-methylhistamine excretion during a long-term follow up of patients with inactive Crohn`s disease
Kimpel S, Nägel A, Backhaus B, Straube S, Buchwald W, Schultis W, Kressel J, Hahn EG, Raithel M

P38: Second-generation antihistamines (SGAs): a systematic review of their effect on quality-of-life (QoL) in intermittent allergic rhinitis (IAR)
Golightly LK

P39: Do both systemic and topical anti-allergy treatments cause ocular dryness?
Gomes P, Abelson MB, Torkildsen G

P40: Use of rEV131 for prevention of anterior chamber inflammation in an animal model
Gomes P, Lane K, Abelson MB, Weston-Davies W

P41: Lidocaine protein binding time response to diphenhydramine administration in rat tissues.
Tiqka E, Daskala I, Tsagatakis M, Melakopoulos I, Saranteas Th, Tesseromatis C

11.30-12.00 Poster session 6: Histamine in Reproduction and Cancer
Chairpersons: *Prof Elena S Rivera & Prof Danuta Maślińska*

P42: Locally secreted histamine may regulate the development of ovarian follicles by apoptosis
Szukiewicz D, Klimkiewicz J, Pyzlak M, Szewczyk G, Maślińska D

P43: Histamine H_4 receptors in human placenta in diabetes-complicated pregnancy.
Szewczyk G, Maślińska D, Szukiewicz D, Deregowski K, Smiertka W, Klimkiewicz J, Pyzlak M

P44: Histamine increase the invasive potential of human melanoma cells
Pocza P, Kovacs P, Pallinger E, Pocza K, Kohidai L, Falus A, Darvas Z

P45: Endogenous and exogenous histamine affects on growth of mice mammary adenocarcinoma
Hegyesi H, Molnár V, Fulop KA, Falus A

P46: Nitric oxide involvement in histamine-mediated PANC-1 cells growth
Cricco GrP, Medina VA, Núñez MA, Mohamad NA, Gutiérrez AS, Sanbuco LA, Bergoc RM, Rivera ES, Martín GA

P47: Differential modulation of the cellular stress response by histamine and the H_1, H_2 receptor antagonists in eukaryotic cells
Delitheos V, Papamichael K, Tiligada E

12.00-13.30 The Art Hancock Young Investigator Award Symposium
Chairpersons: *Prof Walter Schunack & Prof Pertti Panula*

12.00-12.15 O20: Oligomerization of the human histamine H_4 receptor
van Rijn RM, Chazot PL, Shenton FC, van Marle A, Bakker RA, Leurs R

12.15-12.30 O21: Homology modeling of the human histamine H_4 receptor and its application in structure-based drug design
Kiss R, Jelinek I, Falus A, Noszál B

12.30-12.45 O22: Characterization of 4-methylhistamine and VUF 8430 as selective histamine H_4R agonists
Lim HD, Guaita E, Bakker RA, Coruzzi G, Thurmond RL, Leurs R

12.45-13.00 O23: Histaminergic system shapes network synchonization in the hippocampus and striatum in the behaving rat
Ponomarenko AA, Unterbrink D, Li J-S, Korotkova TM, Haas HL

13.00-13.15 O24: Enhanced CREB activity and adipogenic potential in histamine free embryonic fibroblasts
Hegyi K, Falus A, Toth S

13.15-13.30 O25: Excitation of aminergic neurons by histamine
Korotkova TM, Sergeeva OA, Ponomarenko AA, Haas HL

13.30-14.30 Lunch

14.30-17.00 The Art Hancock Memorial Symposium
Chairpersons: *Dr Timothy A Esbenshade & Prof Helmut L Haas*

14.30-14.45 Art Hancock: the man, his achievements and his life at the *EHRS*
Timothy A Esbenshade

14.45-15.00 O26: Biochemical and pharmacological evidence for homo- and heter-oligomeric human H_3 receptor isoforms: further evidence of dominant negative role for splice isoforms
Chazot PL, Shenton FC, van Rijn RM, Bakker RA, Leurs R

15.00-15.15 O27: New signaling pathways for the histamine H_3 receptor
Bongers G, van Marle A, Navis M, Bakker RA, Leurs R

15.15-15.30 O28: Histamine H_3 receptor expression is increased in the brains of transgenic mice over-expressing beta amyloid
Ning X, Liu D, Pong K, Pangalos M, Reinhart P, Hirst W

15.30-15.45 O29: Different response to GABAA or H_3 antagonists suggest the existence of distinct subpopulations among histaminegic neurons
Giannoni P, Cenni G, Passani MB, Mannaioni PF, Medhurst AD, Blandina P

15.45-16.00 O30: Cloning and expression of histamine H_1, H_2 and H_3 receptors in zebrafish and effects of their ligands on behavior
Panula P, Peitsaro N, Sundvik M

16.00-16.15 O31: A new family of histamine H_3 receptor antagonists based on a natural product: discovery, development of SAR, and properties of the series
Cowart M, Sun M, Zhao C, Witte DG, Miller TR, Krueger KM, Browman K, Fox GB, Esbenshade TA, Hancock AA

16.15-16.30 O32: Histamine H_3 antagonists with serotonin reuptake transporter inhibitor activity
Letavic MA

16.30-16.45 O33: Pharmacological classification of histamine H_3 receptor agents across species is attributable to TM3 sequence differences
Gibbs BF, Estvander BR, Miller TR, Baranowski JL, Sharma R, Hancock AA, Krueger KM

16.45-17.00 O34: Fluorophore-tagged non-imidazole histamine H_3 receptor ligands with subnanomolar affinities
Amon M, Ligneau X, Schwartz JC, Stark H

17.00-18.00 Coffee break

17.15-17.50 **Poster session 7: Immunology**
Chairpersons: *Dr Bernhard F Gibbs & Dr Elke Schneider*

P48: *Whithdrawn*

P49: Histamine H_1, H_2 but not H_4 receptors are upregulated during bone marrow regeneration
Horváth Z, Pállinger E, Horváth G, Jelinek I, Falus A

P50: Unexpected action of histamine on dendritic cells under *acidosis*
Amaral MM, Davio C, Martínez D, Geffner J, Vermeulen M

P51: Tissue-related immune-endocrine interactions in experimental hyperthyroidism
Kakavas S, Zampeli E, Delitheos V, Tiligada E

P52: Histamine stimulates electrogenic ion transport in avian epithelia
Collins CB, Campion DP, McGrath J, Baird AW

P53: Peripheral but not central effects of allergic reaction are mediated by histamine
Gerencsér AS, Kovács KJ

P54: Study of adipogenesis in genetically histamine free (HDC KO) and wild type bone marrow derived mesenchymal stem cells
Lux A, Skard I, Toth SP, Falus A

17.15-17.50 **Poster session 8: Receptors and other subjects**
Chairpersons: *Prof Tatjana Irman-Florjanc & Prof Emanuela Masini*

P55: Internalization and resensitization mechanisms of the histamine type 2 receptor
Fernández N, Monczor F, Notcovich C, Baldi A, Davio C, Shayo C

P56: Strain specific differences in Oct4 expression of HDC KO and wild type ES cells
Toth SP, Gocza E, Carstea BV, Falus A

P57: Comparison of the *in vitro* and *in vivo* H_2-H_4 receptor selectivity of 4-methylhistamine and VUF8430
Adami M, Guaita E, Lim HD, Bakker RA, de Esch IJP, Leurs R, Coruzzi G

P58: Porcine plasma amine oxidase has a broad substrate specificity and efficiently converts histamine
Feurle J, Schwelberger HG

P59: Influence of amitriptyline on central histamine-induced reversal of haemorrhagic shock in rats
Jochem J, Irman-Florjanc T, Zwirska-Korczala K

P60: Altered inflammatory gene expression in genetically H_4 histamine receptor deficient mice
Fulop AK, Ribita D, Falus A

P61: Involvement of the histaminergic system in the central cardiovascular regulation in haemorrhage-shocked rats with portocaval anastomosis; preliminary data
Jochem J, Fogel AW, Maksymowicz M, Zwirska-Korczala K, Lewinski A

20.15-22.00 Dinner

Saturday 13 May 2006

08.30-13.00		Registration Desk Open
08.30-09.15	I3:	**Invited lecture** Occupational asthma: diagnosis and management Piero Maestrelli, Italy Introduced by *Prof Pier Francesco Mannaioni*
09.15-10.30		**The D Varonos Memorial Symposium** **"Clinical implications – histamine and disease"** Chairpersons: *Prof Pier Francesco Mannaioni & Dr Catherine Tiligada* *Sponsored by GlaxoSmithKline (Greece)*
09.15-09.30	O35:	The histamine H_4 receptor mediates allergic airway inflammation *Thurmond RL*
09.30-09.45	O36:	Distribution pattern of histamine H_4 receptor in human synovial tissue from patients with rheumatoid arthritis *Jablonowska M, Grzybowska-Kowalczyk A, Wojtecka-Lukasik E, Maślińska D, Gujski M, Maśliński S*
09.45-10.00	O37:	Theophylline as "add-on" therapy to cetirizine in patients with chronic idiopathic urticaria: a randomized, double-blind, placebo-controlled pilot study *Makris M, Kalogeromitros D, Kempuraj D, Katsarou-Katsari A, Grigoriou S, Theoharides TC*
10.00-10.15	O38:	Biphasic immunomodulating effect of cepharolsporin derivatives in man *in vitro* and *in vivo* *Assem ESK, Ezeamuzie CI, Vickers MR*
10.15-10.30	O39:	On the mechanism of antiphagocyte-antioxidative effect of H1-antihistamines *Nosal R, Drabikova K, Jancinova V, Pecivova J, Macickova T, Holomanova D*
10.30-11.00		Coffee break
11.00-12.50		**Oral session 5: Neuro-immuno-endocrine aspects of histamine** Chairpersons: *Prof Wilfried Lorenz & Dr Robin Thurmond*
11.00-11.35	O40:	Evidence of human mast cell function in inflammatory diseases *Theoharides TC, Cao J, Papadopoulou N, Kempuraj D, Tagen M*
11.35-11.50	O41:	*Whithdrawn*
11.50-12.05	O42:	Skin histidine decarboxylase (HDC) and corticotropin-releasing hormone receptor-1 (CRH-R1) genes are overexpressed in chronic urticaria (CU) *Kalogeromitros D, Papadopoulou N, Staurianeas NG, Tiblalexi D, Theoharides TC*
12.05-12.20	O43:	Role of histamine in ghrelin-induced gastroprotection against acute gastric lesions *Konturek PC, Brzozowski T, Pajdo R, Konturek SJ, HahnEG, Raithel M*

12.20-12.35	O44:	Gastric effects of the histamine H_4 receptor antagonists JNJ7777120 and VUF6002 *Coruzzi G, Adami M, Guaita E, de Esch IJP, Leurs R*
12.35-12.50	O45:	G protein receptor kinases (GPKs) regulate histamine H_2R desensitization in human leukemic cells *Fernández N, Monczor F, Tubio MR, Baldi A, Shayo C, Davio C*
13.00-14.00		Lunch
14.00-16.30		**The EUBREN Basophil network symposium** Chairpersons: *Dr Franco Falcone & Prof Clemens Dahinden*
14.00-14.10		Introduction to EUBREN *Franco Falcone*
14.10-14.30	O46:	Control of basophil functions through histamine and other biogenic amines *Schneider E, Machavoine F, Pléau J-M, Ohtsu H, Watanabe T, Schinkel AH, Dy M*
14.30-14.50	O47:	*In vitro* and *in-vivo* stimulatory potential of bone marrow-derived mast cell on tumor growth *Hegyesi H, Darvas Z, Wiener Z, Molnár V, Pos Z, Falus A*
14.50-15.10	O48:	Substantial differences in the kinetics of histamine release from human basophils caused by varying strengths of IgE-dependent activators *Gibbs BF, Räthling A, Zillikens D, Huber M, Haas H*
15.10-15.30	O49:	The IL-4-inducing principle from *S. mansoni* eggs (IPSE) activates human basophils *via* a novel mechanism: "IgE receptor engagement without crosslinking" *Blindow S, Schramm G, Gronow A, Manske M, Galle J, Grevelding CG, Weimar T, Sutton BJ, Gibbs BF, Doenhoff MJ, Haas H*
15.30-15.50	O50:	Accumulation and activation of basophils in allergic disease *Walls AF, Mochizuki A, McEuen AR*
15.50-16.10	O51:	Old and novel mediators of Th2-type immune responses and allergy produced upon mast cell – basophil interactions *Dahinden CA, Spiegl N, Tschopp CM, Didichenko S*
16.10-16.30	O52:	"British diet on a chip": A novel diagnostic tool for the detection of food allergy combining protein microarrays with human basophils *Lin J, Schramm G, Haas H, Falcone FH, Alcocer MJC*
16.30-17.00		Coffee break
16.30-17.00		Poster evaluation Committee
17.15-19.00		**General assembly**
20.30		Farewell Dinner

Sunday 14 May 2006

Check out, Departure

INVITED LECTURES

I 1

Histamine and the EHRS - What Has Happened in the Last 35 Years?

Madeleine Ennis
Respiratory Research Group, The Queen's University of Belfast, Belfast BT12 6BJ, UK

Histamine has a long history compared to many other mediators. In 1907 it was synthesized by Windaus and Vogt and in 1910 it was discovered in nature (Ackermann and Dale & Barger). The idea of a histamine society began in 1971, at a histamine satellite meeting in Lodz organised by the late Czeslaw Maslinski (one of our few Honorary Members). The first official meeting of the then named "Histamine Club" took place in Paris in 1972. This was the same year as Sir James Black (another of our honorary members) and colleagues described the discovery of the H_2 receptor. In the intervening years since that very first meeting, the number of known histamine receptors has increased to four. We now realise that histamine also acts as a modulator of the immune system and is involved in angiogenesis, cancer as well as the allergic diseases. In this talk I will try and highlight some of the important presentations from our meetings showing how the histamine field has developed.

One of the characteristics of our society is its friendliness and the fact that we have enough time for informal contacts. These are really important and often lead to some fantastic scientific collaborations. We also learn something about the countries we visit (so far 16) during our outings and again have the opportunity to network with others.

In the intervening years, we have moved from the Histamine Club to the European Histamine Research Society – complete with a registered address in Belgium and statutes in French as well as members from such "well-known European Countries" such as Argentina, Canada, China, Japan, Mexico and USA (nb this is not an exhaustive list). Last year, Professor Takehiko Watanabe became our first non-European Honorary Member of the Society.

My talk will also be illustrated with photos from as many meetings as possible!

Histamine and Systems Biology; Genes and Genomics Beyond Genes

Andras Falus
Department of Genetics, Cell and Immunobiology, Semmelweis University, Nagyvarad ter 4, 1089 Budapest, Hungary

Systems biology is the study of living organisms in terms of their underlying network structure rather than simply their individual molecular components. A "system" can be anything from a gene regulatory network to a cell, a tissue, or an entire organism. Because systems biology requires investigation of all interacting components simultaneously, high-throughput, quantitative technologies are essential. Computational approaches are also required to handle and interpret the volumes of data necessary to understand complex biological systems.

Histamine biology, pharmacology and medicine provide eminent examples for the need for multilateral approaches. This colorful scenario is traditionally represented in sessions of European Histamine Research Society. SNP and SNP haplotype studies, gene expression and proteomic analysis are currently complemented with metabolomic approaches as well as computational analysis from allergy databanks, since computational models are important complementary methodologies to experimental research. They are particularly useful in fields requiring a large number of experiments due to the combinatorial nature of the underlying systems and processes. In such fields, computational models evolve with the amount of data and the accumulation of knowledge.

Further and even more challenging perspectives are seen on the field of non-coding DNA (PostGenes) or as called earlier the „junk DNA". It turned out that there is a forty times larger difference between the DNA of the chimp and the human in the 98.7% "junkDNA" as compared to 1.3% encoding for proteins. Although the relevance is largely unclear, one of the functions of the PostGene part of genome is coding microRNA hairpins with outstanding, newly recognized importance in gene regulation. In histamine genomics there are fairly good indications, that further attention will be paid to the non-coding PostGenes providing new *haploidomics* predictive and thus experimentally supportable or refutable theories in histaminology.

I 3

Occupational Asthma: Diagnosis and Management

Piero Maestrelli
Department of Environmental Medicine and Public Health, University of Padova, Padova, Italy

Introduction

Occupational asthma (OA) is defined as a type of asthma that is caused by exposure to a product present in the workplace. To fulfill this definition, the causative agent(s) should be almost exclusive to the workplace, hence this definition excludes asthma triggered by physical agents such as cold air or exercise. Besides OA with a latency period for which an allergic mechanism can be identified or is highly probable, another form of work-related airflow obstruction and bronchial hyperresponsiveness has also been described, i.e. irritant-induced asthma or reactive airway dysfunction syndrome (RADS), which may be developed after acute exposure to high concentrations of irritant gases or fumes. RADS therefore develops without a latency period of exposure and, although its functional characteristics resemble those of OA, its symptoms cannot be reproduced by re-exposure of the affected patients to nonirritant amounts of the offending agent.

OA may be induced by several different mechanisms. Immunological mechanisms are generally implicated when OA occurs upon exposure to an agent after a latent period of sensitization. These mechanisms can be further divided into those that induce asthma through either an IgE-dependent or an apparently non-IgE-dependent mechanism. In the latter, specific IgE antibodies are not detectable or are found only in a small proportion of the patients with proven disease, even though the clinical picture is compatible with an 'allergic' reaction (i.e. sensitization and an exaggerated tissue damaging response upon re-exposure). Nonimmunological mechanisms are implicated when OA occurs without a latency period of exposure, i.e. RADS or irritant-induced asthma.

The causes of OA can be classified into high- and low-molecular-weight compounds. High-molecular-weight compounds, which are often from biological sources, generally induce asthma through an IgE-dependent mechanism, whereas the majority of low-molecular-weight compounds induce asthma through non-IgE-dependent mechanisms.

Clinical history

Since an attributable risk of asthma due to occupation of 15% among all asthma cases has been estimated, every subject with asthma should be questioned about his or her current and past workplaces as persistent asthma can be attributed to past exposure. Two clues in the patient's history should point to the possibility of OA: the symptoms, and the job and products at work. First, the subject may have a clear-cut history of exacerbations of respiratory symptoms at work. Secondly, the job and product at work can suggest the diagnosis. The nature of all products present in the workplace, not only those handled by the subject, should be obtained by requesting safety data sheets. There could be products present that have not been listed as known causes of OA, but this does not preclude the possibility of there being so. At the onset of symptoms, improvement at weekends and when on vacation is generally the rule, although eventually the symptoms will persist through these short periods away from work.. It must be remembered that questionnaires are sensitive, but that they are not specific tools, when results are compared with the final diagnosis.

Immunological assessment

Specific IgE or IgG antibodies against an occupational sensitizer have been detected mainly for high-molecular-weight agents. The value of in vivo or in vitro tests in establishing a sensitization to low-molecular-weight chemicals has proved limited. In addition, the presence of immediate skin reactivity or increased specific IgE or IgG may reflect exposure or sensitization but it does not imply that the target organ is involved. With high-molecular-weight allergens, negative skin tests to such allergens almost completely exclude the possibility of OA. With low-molecular-weight allergens, such as isocyanates and red cedar, negative skin tests or specific IgE or IgG do not refute or confirm the diagnosis of OA; skin tests are also usually unavailable.

Specific antibodies to allergens may be demonstrated in biological fluids using a variety of tests. They confirm a sensitization demonstrated by skin test but are often less sensitive. They represent an alternative to the skin test when the preparations of antigens have irritant, toxic, or mutagenic effects, and in patients under pharmacological treatment that blunts normal skin reactivity. Control for specificity is required, especially when protein conjugates are used. Different factors, such as total IgE level, characteristics of the conjugate, carrier specificity, and cross-reactivity with other antigens, may affect the results. Assessment of the chemokine monocyte chemoattractant protein-1 (MCP-1) produced in vitro by diisocyanate-stimulated blood mononuclear cells exhibited higher test efficiency than specific antibodies for identification of isocyanate asthma. Other in vitro tests, such as histamine release from basophils, are less standardized but may be useful occasionally.

Physiological assessment

The presence of airway obstruction with demonstrable reversibility after inhaling a bronchodilator is a well-recognized confirmatory step for asthma. If there is no significant airway obstruction, the demonstration of increased bronchial responsiveness is suggestive of asthma, not necessarily of OA.

Serial PEF monitoring has been proposed for both the investigation and assessment of asthma. The sensitivity and specificity of PEF monitoring, as compared with the 'gold standard' specific inhalation challenges, varies from 72% to 89% depending on the study. Combining PEF and the assessment of eosinophils in induced sputum for periods at work and away from work may improve the diagnostic yield.

Laboratory challenges in small cubicles were proposed by Pepys in the 1970s in an effort to reproduce the workplace environment. Improvement in the methodology of the test has been put forward using closed-circuit apparatus for dry particles and vapors, including isocyanates. After exposure, various temporal patterns of reactions can occur, including those of typical (immediate, late, and dual) and atypical reactions (progressive, square waved, and prolonged immediate).

Outcome

The outcome of OA after diagnosis is often poor. Removal from the occupational exposure is associated with recovery from asthma in about 50% of subjects. Many retrospective studies have unanimously demonstrated a persistence of asthmatic symptoms, bronchial obstruction, and hyperresponsiveness in subjects with OA after being removed from exposure. Most studies also showed that the total duration of exposure, the duration of exposure after the onset of symptoms, and the severity of the asthma at the time of diagnosis are all determinants of the prognosis. Improvement in bronchial responsiveness occurs predominantly in the first two years after cessation of exposure and continues, though at a slower rate, later on. If exposure continues, there is overall deterioration in the asthmatic condition.

Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Am J Respir Crit Care Med 2005;172:280-305

ORAL COMMUNICATIONS

O 1

Novel Ligands Stabilize Stereo-Selective Conformations of the H_1 Receptor to Result in Functionally-Selective Activation or Antagonism of Intracellular Signaling Pathways

Raymond G Booth
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Depending on its conformation, the histamine H_1 GPCR can couple to several different G protein types to activate multiple intracellular signaling pathways. We developed novel ligands that stabilize stereo-selective conformations of the H_1 receptor to result in functional selectivity. The cyclic tertiary amines, (±)-c*is*-5-phenyl-7-dimethylamino-5,6,7,8-tetrahydro-9*H*-benzocycloheptane (*cis*-PAB) and (–)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (*trans*-PAT), are functionally-selective agonists at H_1 receptors, stimulating phospholipase (PL) C/inositol phosphate (IP) and adenylyl cyclase (AC)/cAMP signaling, respectively, in clonal cells and mammalian tissues. *Cis*-PAB and *trans*-PAT also are functionally-selective H_1 antagonists of AC/cAMP and PLC/IP signaling, respectively. Molecular determinants for *cis*-PAB *vs. trans*-PAT conformationally-selective binding to H_1 receptors, that leads to functionally-selective effects on PLC/IP vs. AC/cAMP signaling, likely involves stereochemical factors, as well as, more subtle steric influences. In bovine adrenal cells, tyrosine hydroxylase (TH) and catecholamine synthesis is activated (EC50 +/- SEM, µM; Emax +/- SEM at 10 µM, % control) by histamine (0.16 +/- 0.08; 200+/- 4), *cis*-PAB (1.0 +/- 0.1; 160 +/- 14), and *trans*-PAT (1.4 +/- 0.1; 160 +/- 5.3) in a manner blocked by H_1 antagonists. Inhibition of PKA, but, not PKC, abolishes the histamine and *trans*-PAT effect, suggesting H_1 agonism by these ligands exclusively activates AC/cAMP/PKA signaling to stimulate TH. Inhibition of PKA or PKC abolishes the *cis*-PAB effect. Since PAB activation of H_1 receptors selectively stimulates PLC/IP signaling (antagonizes AC/cAMP signaling), it appears H_1/PLC/IP/PKC signaling can activate PKA, downstream of cAMP production, to stimulate TH. Thus, there appears to be PKC to PKA signaling “cross-talk” that can confound prediction of functional selectivity effects downstream of second messenger formation. Support: NIMH 068655.

O 2

Fast Production and Analysis of the Human Histamine H_1 Receptors Using a Cell Free Protein Expression System Followed by MS Analysis

Kamonchanok Sansuk[1], Remko Bakker[1], Paul Hensbergen, Rob Leurs[1]

[1]Leiden/Amsterdam Center for Drug Research (LACDR), Vrije Universiteit Amsterdam, Department of Pharmacochemistry, De Boelelaan 1083, 1081 HV, Amsterdam; [2]Biomolecular Mass Spectrometry Unit, Leiden University Medical Center (LUMC), Department of Parasitology, P.O. box 9600, 2300 RC, Leiden, The Netherlands

Rational design of ligands for G protein coupled receptors (GPCRs) relies for a large extent on receptor models. Computer models of GPCRs, however, are commonly based on bovine rhodopsin for which a high resolution 3D GPCR structure is available, in conjunction with site-directed mutagenesis data for the GPCR at hand. In order to produce better models structural data of the studied receptor would be needed. One of the requirements for structural studies is to have access to large amounts of pure GPCR protein.

Using a baculovirus expression system we previously successfully produced large amounts of the human histamine H_1 receptor (hH_1R) (Ratnala VRP et al., *Eur. J. Biochem*. 2004; 271:2636-2646). Here we have employed a cell free expression system to produce large quantities of the hH_1R. We have tried to optimize solubilization, purification and reconstitution of the produced hH_1R for subsequent structural analysis. Using MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time Of Flight) mass spectrometry we were able to detect a large percentage of the hH_1R consisting of both intra- and extracellular loops, amino- and carboxyl-termini as well as transmembrane domains. The use of a cell free expression system enables a much faster production of protein compared to baculovirus expression, whereas MS analysis requires much smaller amounts of protein for analysis compared to solid state NMR. In summary, the cell free expression combined with MS structural analysis enables a fast and specific screening of the target receptor.

This work was in part supported by NIH grant R01 MH068655.

O 3

Effect of Sulphasalazine and Balsalazide on Histamine Release from Mast Cells

Kheng H Peh[1], Beatrice YC Wan[1], El-Sayed K Assem[2], Frederick L Pearce[1]
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Sulphasalazine (SAZ), balsalazide (BSZ) and their active moiety, 5-aminosalicyclic acid (ASA) offer effective treatment of ulcerative colitis. SAZ, BSZ and ASA inhibit histamine release (HR), the proinflammatory mediator, from mast cells (MC) [1,2]. We have studied the effect of SAZ, BSZ and ASA on HR from RBL-2H3 cells (mucosal type MC) and rat peritoneal MC (RPMC, connective tissue type MC).

RBL-2H3 cells [3] were pre-incubated with 5, 50 and 500 μM of test compounds for 30 min, followed by the addition of submaximal concentration of Con A (concanavalin A, 50 μg/ml, immunological stimulant), NaF (20 mM, non-specific G-protein activator), PLC (0.08 U/ml, phospholipase C) or A23187 (1.0 μM, calcium ionophore) for 15 or 30 min. For experiments with RPMC, cells from naive or *Nippostrongylus brasiliensis*-sensitised Sprague-Dawley rats (250-350 g) were preincubated with test compounds (0.16–500 μM) for 0 or 5 min prior to the challenge with anti-IgE serum (1/250 dilution), Con A (4 μg/ml), NaF (20 mM), PLC (0.08 U/ml), A23187 (10 μM) or DiC8 (PKC activator, 20 μM) for 10, 15 or 60 min [3].

With RBL cells, only NaF-induced HR was reduced by SAZ (51%, $p<0.01$) and BSZ (32%, $p<0.05$) at 500 μM but not ASA. No other induced HR was inhibited. Inhibition of Con A-induced HR by H_2O_2 (1 mM) was not reversed by SAZ, BSZ or ASA.

With RPMC, anti-IgE-induced HR was inhibited slightly by SAZ (at 20 μM, 12%, $p<0.01$) and markedly by BSZ (at 20μM, 48%, $p<0.01$; at 100 μM, 39%, $p<0.05$) but potentiated slightly by ASA (at 100–500 μM, 10–17%, $p<0.05$). Marked and dose-related inhibition of Con A-induced HR was shown by SAZ (at 20–500 μM, 41–79%, $p<0.05$, or <0.01) and BSZ (at 4–500 μM, 51–77%, $p<0.01$). NaF-induced HR was only inhibited by SAZ (24%, $p<0.001$) and ASA (14%, $p<0.01$). No other induced HR was inhibited.

With the exception of part of the results with NaF (RPMC), inhibition of induced HR by SAZ BSZ and ASA in the two cell preparations seems to be due to modulation of G-protein-controlled transmembrane signaling via diverse pathways.

[1] Peh KH et al. Br J Pharmacol 1990;100:449P
[2] Bissonnette EY et al. J Immunol, 1996;156:218-23
[3] Wan BYC et al. Biochem Pharmacol 2001;62:1537-44

Luminometric Determination of Amine Oxidase Activity

Hubert G Schwelberger, Johannes Feurle
Labor für Theoretische Chirurgie, Universitätsklinik für Chirurgie, Medizinische Universität Innsbruck, Austria

Amine oxidases play a key role in the inactivation of histamine. Diamine oxidase (DAO), a secreted, soluble copper-containing amine oxidase, catalyzes the direct oxidative deamination of histamine probably in the extracellular space. FAD-containing monoamine oxidases (MAO) of the outer mitochondrial membrane have been implicated in the oxidation of methylated histamine produced by histamine N-methyltransferase inside cells. Although numerous assays have been described for measurement of amine oxidase activity these either suffer the limitation of low sensitivity or require the use of certain amine substrates. Therefore, a new type of assay was developed that is not only extremely sensitive but also allows the measurement of the conversion of any amine substrate by any amine oxidase. In this assay, hydrogen peroxide, the common product of all amine oxidation reactions, is used by horseradish peroxidase to oxidize luminol in the presence of enhancers, which produces light that can be measured in a luminometer. This assay allows the simple assessment of substrate specificity, determination of kinetic parameters, study of enzyme inhibition mechanisms and investigation of long-term enzyme stability. The assay can be performed in single tube or microplate format and is very cost-efficient due to negligible reagent consumption.

O 5

Activation of Cannabinoid Receptors Prevents Allergen-Induced Asthma-Like Reaction in Sensitized Guinea Pigs

Francesca Fabrizi[1], Alfredo Vannacci[1], Lucia Giannini[1], Chiara Mariottini[1], Beatrice Passani[1], Pier Francesco Mannaioni[1], Silvia Nistri[2], Emanuela Masini[1]
[1]Department of Preclinical and Clinical Pharmacology; [2]Department of Anatomy, Histology and Forensic Medicine, University of Florence, 50139 Florence, Italy

Asthma is defined by airway inflammation and hyperresponsiveness, and its morbidity has increased worldwide. The bronchodilating properties of Cannabis Indica derivatives are well known and recently the antinflammatory activity of CB_2-receptor agonists in isolated guinea pig mast cells has been shown.

This study evaluates the effect of a synthetic CB receptor agonist (CP55,940) on asthma-like reactions to inhaled antigen in actively sensitized guinea pigs *in vivo*. Male albino guinea-pigs, sensitized to ovalbumin, placed in whole body respiratory chambers, were challenged with ovalbumin. CP55,940 (0.4 mg/Kg b.w.) were given i.p. 3 hours before ovalbumin challenge. Some animals, 30 min before CP55,940 treatment, received i.p. a CB_1 (AM251, 0.1 mg/Kg b.w.) or a CB_2 –receptor antagonist (SR144528, 0.1 mg/kg b.w.).

The following functional parameters were examined: latency time for the onset of respiratory abnormalities, cough severity score, occurrence and duration of dyspnoea. We also evaluated lung tissue histopathology, mast cell degranulation, lung tissue concentration of myeloperoxidase, malonyldialdehyde and 8-hydroxy-2-deoxyguanosine, as well as manganese superoxide dismutase and prostaglandin D_2 in the bronchoalveolar lavage fluid (BAL).

Both respiratory abnormalities and bronchoconstriction in response to ovalbumin challenge are nearly absent in naïve animals, while they sharply became severe in sensitized animals. Treatment with CP55 significantly reduced the severity of cough and the occurrence of dyspnoea, and delayed the onset of respiratory abnormalities. Furthermore, all the biochemical changes induced by ovalbumin in lung tissue or BAL fluid were prevented by CP55 treatment. The pre-treatment of sensitized animals with CB_1 (AM251) and CB_2 (SR144528) –receptor antagonists reverted the protective effects of CP55, indicating that both CB_1 and CB_2 receptors are involved.

These results show that the cannabinoid receptor agonist CP55 can counteract acute allergic asthma-like reaction in actively sensitized guinea-pigs and suggest a possible future use of cannabinoid agonists in asthma.

Effects of the Carbon Monoxide Releasing Molecule CORM-3 in a Coincubation Model with Rat Mast Cells and Human Neutrophils

Alfredo Vannacci, Lucia Giannini, Francesca Fabrizi, Caterina Uliva, Rosanna Mastroianni, Emanuela Masini, Pier Francesco Mannaioni
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The heme oxygenase (HO) enzymes are able to split the tetrapyrrole heme ring to biliverdin, free ferrous iron, and carbon monoxide (CO). At least two isoforms of heme oxygenase are expressed in mammalian cells: HO-1 (inducible isoform) and HO-2 (constitutive). In particular, HO-1 is a stress-responsive enzyme that acts during inflammatory reactions, regulating immunological responses involved in cardiac anaphylaxis, in allergic reactions and in the rejection of transplanted organs. Previous reports from our group showed that exogenous CO or water-unsoluble CO-releasing molecules were able to mimic the anti-allergic and anti-anaphylactic effects of HO-1 in isolated guinea pig hearts, in guinea pig mast cells and in human basophils, mainly through the activation of the soluble guanylyl cyclase. Here we report the effects of the water soluble CO-releasing molecule CORM-3 in a coincubation of rat serosal mast cells (MCs) and human neutrophils (PMN), activated with the chemotactic peptide *formyl-methionyl-leucyl-phenylalanine* (fMLP, 10 nM). The expression of CD203c upon MC membrane (evaluated through flow cytometry) as well histamine release (fluorimetric method) were increased after the incubation with PMN stimulated by fMLP. CORM-3 (100 nM-10 uM) was able to reduce the activation of MCs, while the inactivated form of the drug (iCORM), unable to release CO, was ineffective. PMN significantly increased the production of ROS upon activation with fMLP and, consistently with the hypothesis that superoxide anion plays a role in MC activation, the treatment of the cells with SOD (300 IU/ml) mimicked the effects of CORM-3. Finally, CORM-3 also reduced the activation of human PMN, assessed as the membrane expression of CD11b (evaluated through flow cytometry). The inactivated form of CORM-3 and SOD were ineffective.

In conclusion, CORM-3 was effective in reducing fMLP-PMN-induced MC activation. The effect was mediated by the release of CO, since the iCORM was ineffective. We can also suggest an involvement of superoxide anion, since the activation of MCs was reverted incubating the cells with SOD.

O 7

Prevention of Antigen-Induced Bronchoconstriction by Epigallo-catechine-3-Gallate in Actively Sensitized Guinea Pigs

Lucia Giannini[1], Caterina Uliva[1], Daniele Bani[2], Rosanna Mastroianni[1], Ylenia Suzuki[3], Silvia Nistri[2], Pier Francesco Mannaioni[1], Hisanori Suzuki[4], Emanuela Masini[1]

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It has been showed that nitric oxide (NO) plays a role in asthma, even if the pathophysiological connection between NO and asthma remains uncertain. In this study we used an animal model of asthma-like reaction, to provide insight into the possible role of NOS-derived NO in the pathophysiology of early asthma and to test the possible therapeutic effect of epigallocatechin-3-gallate (EGCG), a polyphenol present in green tea that enhances NO synthase (NOS) activity. For comparison, we used epicatechine (EC), which shares antioxidant but not NOS-modulating properties with EGCG.

Ovalbumin-sensitised guinea pigs placed in a respiratory chamber were challenged with ovalbumin. EGCG (25 mg/kg b.wt.) or EC (25 mg/kg b.wt.) were given i.p. 30 min before ovalbumin challenge. We analysed latency time for the onset of respiratory abnormalities; cough severity; duration of dyspnoea; lung tissue histopathology; mast cell activation (by granule release); leukocyte/eosinophilic infiltration (by major basophilic protein and myeloperoxidase); oxygen free radical-mediated injury (by nitrotyrosine and 8-hydroxy-2-deoxyguanosine); NOS activity; bronchial inflammatory response (by TNF-α in bronchoalveolar lavage, BAL).

Severe respiratory abnormalities appeared in the sensitised animals soon after the antigen challenge, accompanied by bronchoconstriction, alveolar inflation and a marked increase in the assayed parameters of inflammatory cell recruitment, free radical lung injury and release of proinflammatory molecules in BAL fluid. This was associated with marked depression of constitutive NOS activity. Pretreatment with EGCG, but not EC, significantly reduced all the above parameters and sustained endothelial-type NOS activity.

These findings indicate that EGCG, probably by modulating NOS activity, can counteract allergic asthma-like reactions in sensitised guinea pigs and suggest that it may be useful for the treatment of asthma in the future.

Increased Nitric Oxide Production Mediates T Cell Signal Transduction in Histidine Decarboxylase Knockout Mice

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Histamine is a key regulator of the immune system, increased histamine production is a well known feature of many allergic diseases. Several lines of evidence suggest the role of histamine in T cell activation: T cells express both type 1 and type 2 histamine receptors, histamine inhibits the production of Th1 cytokines such as IL-2 and interferon-γ (IFN) and enhances the secretion of Th2 cytokines. IFN-γ was previously shown to regulate nitric oxide (NO) production. NO is a multifunctional intracellular and intercellular messenger, NO mediates lymphocyte proliferation differentiation and apoptosis. Our previous work indicates that NO is necessary for effective T cell activation.

To study the role of histamine in T cell activation, we investigated T cell signal transduction in histidine decarboxilase knockout (HDC-KO) and wild type mice.

Splenocytes from wild type and HDC-KO mice were isolated following *in vivo* stimulation with complete Freund's adjuvant. NO, cytoplasmic Ca^{2+} concentrations and reactive oxygen intermedier (ROI) levels were determined by flow cytomerty. IFN-γ mRNA level was measured by RT PCR.

Increased INF-γ mRNA level of HDC-KO splenocytes was associated with a markedly increased (2,5 fold, p=0,00086) NO production, compared to splenocytes of wild type animals. Activation of T cells through the TCR initiates a biphasic elevation in cytosolic Ca^{2+} concentration, a rapid initial peak observed within seconds and a plateau phase lasting up to 48 h. In response to Con-A stimulation, rapid Ca^{2+} fluxing was diminished ($p<0.05$) while the plateau phase was increased ($p=0.0024$) in HDC-KO T cells. T cell activation-induced NO and superoxide signals were similar in HDC-KO and wild type mice ($p=0.45$; $p=0.23$ respectively).

Our present data support the effect of histamine on T cell signal transduction.

O 9

Histamine Influences the DNA Interaction of STATs in Human Lymphocytes

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As signal transduction in thymocytes is correlated to signal transducer and activator of transcription (STAT), and histamine is an important mediator in allergic diseases the question arises whether histamine acting through histamine receptors (HR) induces crosstalk or modulation of the JAK/STAT pathway – especially the STAT1 interaction at the nuclear level.

Phytohemagglutinin (PHA) stimulated PBMC from atopic (n = 4) and non-atopic (n = 3) donors which served as an ex vivo model for measurements of the effect of histamine, clobenpropit (H3R antagonist, H4R agonist), thioperamide (H3R/H4R antagonists) and the neutral H4R antagonist JNJ7777120 on STAT1. DNA-binding was examined by electrophoretic mobility shift assay (EMSA) technique using STAT1 oligonucleotide (5´- CATGTTATGCATATTCCTGTAAGTGAAAA-3´; Metabion, Martinsried, Germany).

In agreement with previous results IL-4 production was increased and IFN-γ was decreased in the atopic as compared to the non-atopic group at the end of the lymphocyte 3-day culture period. This was correlated to down-regulation of STAT1 signalling in atopic individuals. Western blots showed STAT1α (91kDa) and additionally an 118kDa-band from which a 28kDa-fragment was cleaved in both groups.

The addition of histamine or clobenpropit led to a significant suppression of the formation of STAT1α in the non-atopic group. The STAT1α production in this group was enhanced by the H4R antagonists (thioperamide or JNJ7777120). The phosphorylation of STAT1 was also regulated by the H4R, as reflected by EMSA experiments showing decreased DNA-binding in the atopic group. Interestingly, the 28kDa-STAT1 DNA-binding was significantly suppressed by histamine ($p<0.05$), but significantly enhanced by the neutral H4-antagonist JNJ7777120 in both groups ($p<0.001$).

These results suggest that histamine influenced STAT1 downstream and modulated promoter gene interaction of the 28kDa-STAT1-fragment.

Amine System Project

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Histamine, polyamines and other biogenic amines have pleiotropic physiological effects and the impairment of their metabolism is associated with multiple highly prevalent pathological situations [1]. New systemic approaches are needed for a more efficient advance of knowledge in order to control biogenic amine metabolism and its implication in pathophysiological conditions [2].

To achieve this goal, we use a practical approach: to generate an interconnected database to obtain emergent information on the molecular basis of biogenic amine metabolism and functions. We will build a bilingual (English-Spanish) online platform containing three working areas: an ontology-based *integration* area for the available information on the components of the system, a *predictive* and a *training* area.

With respect to the predictive area, we are working on: i) protein modelling, that has allowed us to obtain the first 3D structural model for mammalian histidine decarboxylase [3]; ii) we have also built the first mathematical model integrating polyamine, arginine and sulfur amino acid metabolism (unpublished results). The architecture of the project is available in its provisional web page (http://av.bmbq.uma.es/asp). The results of this pilot project will be opened to students and scientists from other research centres and countries.

In conclusion, our project is expected to contribute to advance in the systemic integration of research on histamine, polyamine and other biogenic amines. European groups working on fully characterized experimental models are strongly encouraged to join us.

[1] Medina et al. Critic Rev Biochem Mol Biol 2003;38:23
[2] Medina et al. J Cell Mol Med 2005;9:854
[3] Moya-García et al () BioEssays 2005;27:57

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O 11

Effects of Histamine on Triglyceride Breakdown in Mouse Adipocytes

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Recent studies led to consider white adipose tissue (WAT) as a potential target for diverse histamine actions: treatments with H_3-antagonists lower body weight gain (Hancock & Brune. Expert Opin Investig Drugs 2005;14:223-41) while histamine-deficient mice exhibit increased adiposity. However, direct histamine effects on WAT are scarcely documented. Several reports have demonstrated that histamine stimulates lipolysis in dog and rat adipocytes via H_2-receptor activation. Other studies have shown that high doses of histamine can induce lipolysis inhibition, as a consequence of amine oxidation by the amine oxidases (AOs) present in adipocytes and subsequent hydrogen peroxide production. The aim of this work was therefore to study the influence of chronic AO blockade on histamine metabolic effects, and to search whether histamine action on lipolysis is altered in obesity. Semicarbazide was administered during 8 weeks in the drinking water of mice in order to totally block copper-containing AOs. Mice rendered obese by high fat diet were also studied, together with their lean controls. Lipolytic responses were studied in adipocytes from intra-abdominal WAT while subcutaneous (sc)WAT served for determination of AO activity. Chronic semicarbazide treatment (100 mg/kg) totally blocked the semicarbazide-sensitive amine oxidase activity (SSAO) in WAT without changing monoamine oxidase (MAO) activity. Treatment lowered body weight gain. Histamine did not activate lipolysis in control adipocytes but was moderately lipolytic in semicarbazide-treated mice. On the contrary, body and WAT weights were increased in diet-induced obese mice while SSAO showed only a slight tendency to be increased in WAT. Histamine (10-100 µM) was unable to stimulate lipolysis in both control and obese mice. These studies demonstrate that, at least in rodents, changes in histamine metabolism can modify histamine action on lipolysis. However, AO expression in adipocytes is not altered enough with diet-induced obesity to influence histamine metabolism and direct control of fat cell lipolysis.

Highlights in Histamine Function and Reproduction

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Histamine seems to have an indisputable role in reproductive functions.

In males histamine influences fertility: gonadal development, spermatogenesis and sexual behavior. In females the role of histamine in the reproductive processes has been described from several aspects. It is required for normal ovulation, for the process of menstrual cycle, for placental blood flow regulation. It regulates the contractile activity of the uterus and lactation as well. Histamine has multiple functions in the process of pregnancy due to its vasoactive, differentiating and growth-promoting characteristics. Histamine is produced either by mast cells and/or by different cells of the reproductive organs. Pre – and postimplantation events are accompanied by high histidine decarboxylase (HDC) enzyme activity. H1, H2 receptors and diamine-oxidase are co-expressed in both decidual and placental cells in humans. Furthermore, the expression of HDC is much higher in the placenta than in any other organ.

Despite these results, exploring the influence of histamine in reproduction has not been a highlighted field. To fill this gap, our group has studied the regulatory role of histamine in reproduction using the HDC knockout in vivo mouse model. We found differences in the androgen production, gonadal development and sexual behavior in males. We measured alterations in the length of the menstrual cycle in histamine deficient mice. We found histamine dependency and differences in placental functions such as steroidogenic enzyme and cytokine expression. We described the importance of histamine in the establishment of proper Th1/Th2/Th3 balance at the placental-maternal interface during pregnancy.

Hereby we intend to present an overall summarizing picture about the influence of histamine in reproduction, underlining its special importance during pregnancy.

O 13

Expression of Histamine H_3 and H_4 Receptors in Benign Lesions and Malignant Carcinomas of the Human Mammary Gland

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We have previously reported that human mammary gland and benign lesions expressed both H1 (H1R) and H2 (H2R) receptors. On the other hand 100% and 75% of breast carcinomas presented H1R and H2R respectively; the absence of H2R seemed to be related with bad prognosis. The aim of present work was to determine the presence of H3 (H3R) and H4 receptors (H4R) in human mammary biopsies and cell lines (HBL-100 and MDA-MB-231). We also studied the expression of histidine decarboxylase (HDC), proliferating cell nuclear antigen (PCNA) and histamine (HA) content. Samples were obtained by surgery from 45-55 year-old women, 5 corresponded to benign lesions and 15 to breast carcinomas or metastasis. HA and proteins were determined by immunohistochemistry using specific antibodies.

Results indicated that HDC expression was detectable in 50% of samples and no correlation was observed either with the other proteins or with tumor characteristics. All samples presented HA and the content was higher particularly in carcinomas and metastasis. PCNA was positive in most biopsies, showing a lower or negative expression in benign lesions and a direct correlation with invasiveness and metastasis. In this regard PCNA and HA content showed the same tendency. With respect to H4R the benign lesions were negative and 50% of carcinomas showed a low positive mark, all of them corresponding to metastasis or high invasive tumors. The H3R were positive in all samples, showing a lower expression in benign lesions. This result was clearly evident in the benign tissue surrounding the carcinomas that in all cases presented a lower or negative expression compared to the carcinoma of the same patient.

This preliminary report is one of the first to describe the presence of H3R in human mammary tissue. In agreement the cell lines studied presented both H3R and H4R. Remarkably their expression was higher than H1R and H2R as determined by Western blot and their presence may be related to cell proliferation.

Generation of the First Anti-hH_4 Receptor Antibody: Identification of the H_4R in Human Lymphocytes and Brain

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The histamine H_4 receptor (H_4R), a prototypical member of the superfamily of G-protein coupled receptors (GPCRs), has been identified recently through the use of bioinformatics by several groups simultaneously. The H_4R expression is almost exclusively restricted to hematopoietic cells, and is thus suggested to mediate functions of the immune system and as such is a target for the development of anti-inflammatory drugs. Recently, based on mRNA studies, it has been suggested that the H_4R is also expressed in particular parts of the human brain, including the basal ganglia.

To probe this possibility further, we have raised a rabbit polyclonal anti-hH_4 (374-390) receptor antibody, which represents the first published selective immunological probe for the hH_4R. Antibody production and affinity purification was performed as previously described (Chazot *et al*., 2001). The selectivity of the anti-hH_4R antibody for recombinant and native (lymphocytes) hH_4Rs was confirmed by blockade with the respective immunogen peptide and a lack of cross reactivity with the recombinant human H_3R, the most sequence-related GPCR. We have used this antibody to study the oligomeric nature of recombinant and native H_4Rs (Van Rijn *et al*., this meeting). Using our new anti-H_4 and anti-H_3R (Chazot *et al*., this meeting) immunological probes, we demonstrate, for the first time, the presence of both H_4R and H_3Rs in human brain putamen.

Therefore, the human putamen co-expresses H_3 and H_4Rs, which emphasizes the importance of subtype selective compounds to define the role of these closely related subtypes in the function of the basal ganglia. The possibility of hH_3H_4 hetero-oligomers should also not be discounted, and warrants further investigation.

Chazot PL, Hann V, Wilson C, Lees G, Thompson CL. NeuroReport 2001;12:259-262

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O 15

Aversive Memory Consolidation and Reconsolidation: Differences in Neuro-transmitter Engagement

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Whether retrieval of a consolidated memory renders it fragile, and reconsolidation is required to keep the original memory are still open questions. We examined whether neuronal systems underlying consolidation in the basolateral amygdala (BLA) are required for recalled memories to persist. Experiments were done in compliance with the EU recommendations. Adult male Wistar rats received an electrical footshock in a conditioning apparatus (TC). After 7 days, rats were returned to the TC and freezing time was measured. A Na-channel blocker, tetrodotoxin (TTX, 5 ng), a H_3 antagonist, thioperamide (44 pg), a muscarinic antagonist, scopolamine (50 μg), or AM251 (280 pg), a cannabinoid receptor 1 antagonist, were injected into the BLA. A 1st group was treated immediately after training on day 1. A 2nd group was injected 4 days after training without being reexposed to the TC, as well as a 3rd group that was reexposed to the TC immediately before drug treatments. In the 1st group ANOVA and "All Pairs Tukey-Kramer" test revealed that all drug-treated groups froze significantly less than controls (saline-injected) ($F(4, 44) = 5.271$; $P < 0.05$). In the 2nd group no drug-treatments caused significant change of freezing duration compared to saline. In the 3rd group AM251- and TTX-injected rats displayed a significant decrease of freezing time compared to all other groups (ANOVA and "All Pairs Tukey-Kramer" test $F(4,51) = 5.582$; $P < 0.05$). This report shows that reactivated fear memories return sensitive to pharmacological treatments that produce amnesia. TTX demonstrates that BLA neuronal activity is required for fear memory consolidation and reconsolidation, but cannabinoids control both processes, whereas cholinergic and histaminergic neurons only consolidation. The lability of retrieved memory affords opportunities to treat disorders, such as posttraumatic stress or chronic pain, and these results help searching for therapeutic targets to erase stubborn memories.

Properties of Native $GABA_A$ Receptors in Histaminergic and Orexinergic Neurons

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An important step in sleep generation is inhibition of the wake-promoting neurons regular firing by GABA, mainly from the sleep-active ventro-lateral preoptic area (VLPO). The $GABA_A$ receptor ($GABA_AR$) is a target for sedative pharmacotherapy especially in the histaminergic tuberomamillary nucleus. Behavioural studies on mice with deleted or mutated $GABA_AR$ subunits indicated that $GABA_ARs$ expressed in the hypothalamus, which contain α2 and β3 subunits are most important for sleep regulation. Neurons were isolated from two major waking centres in the posterior hypothalamus of rats: histaminergic neurons from the tuberomamillary nucleus and orexinergic neurons from the perifornical area. Whole-cell recordings (patch-clamp) were combined with single cell RT-PCR for 13 subunits of the $GABA_AR$. Pharmacological characterization indicates 3 overlapping but not identical functional areas responsible for $GABA_AR$ heterogeneity: i) sensitivity to GABA ($EC_{50}s$ vary from 2 to 60μM, determined by γ-subunit expression), ii) propofol resistance (determined by the ε-subunit expression), iii) expression of constitutively active $GABA_ARs$ of unknown composition. Further heterogeneities are likely to exist. The data are relevant for understanding the role of the histaminergic system in the regulation of sleep and consciousness.

O 17

Characteristics of Cortical EEG and Sleep-Wake Cycle in Histamine H_1-receptor Knockout Mice

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Using knockout (KO) mice lacking histidine decarboxylase (HDC, the histamine synthesizing enzyme), we have shown the importance of histaminergic neurons in maintaining the brain awake faced with behavioral challenges (Parmentier et al., 2002, J.Neurosci.). Since the central action of histamine is mediated by several receptor subtypes, it remains to determine which one(s) could be responsible for such a role. We have therefore compared the cortical EEG, sleep and waking (W) under baseline conditions or following behavioral or pharmacological stimuli in the PCR confirmed wild type (WT) and H1-receptor KO littermates. We found that H1-KO mice shared several characteristics of HDC-KO mice, i.e. 1) no major change in the daily amount of spontaneous W, but a decrease after lights-off; 2) a decreased EEG slow wave sleep (SWS)/W power ratio; 3) inability to maintain W in response to behavioral challenges as demonstrated by a decrease in sleep latencies following a simulation of injection or an environmental change. These effects are likely to be mediated by central H1-receptors. Indeed, in WT mice, injection of brain-penetrating H1-receptor antagonists (e.g. tripolidine, i.p. 1-5 mg/kg), but not that of non brain penetrating ones, increased SWS, whereas injection of ciproxifan (H3-receptor antagonist) elicited W; all these injections had no effect in H1-KO mice. H1-KO mice also showed a greater increase in EEG power (notably 0.8-5Hz band) than that seen in WT mice after injection of muscarinic receptor antagonist (e.g. scopolamine 0.5 mg/kg, i.p). These data suggest that the role played by histamine neurons in maintaining the brain awake face with behavioral challenges is ensured, in part, by H1-receptors and that an up-regulation of cholinergic transmission might explain a quasi-normal daily amount of W seen in KO mice lacking H1-receptors or histamine synthesis.

Histamine and Behavioral State-Dependent Control of Hippocampal Plasticity

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Histamine neurons in the posterior hypothalamus project widely through the whole brain to control behavioral state and homeostatic body functions. The cellular and molecular prerequisites linking histamine to memory functions are largely unknown. Pharmacological and electrophysiological evidence from previous work indicates that histamine via actions on H1-, H2-, H3-, and NMDA-receptors affects the intrinsic properties and transmitter release of glutamatergic and GABAergic neurons, thereby acting as a powerful inductor of synchronous network activity and synaptic plasticity in the hippocampus. We have begun to further delineate the role of histamine in neuronal plasticity using histamine-deficient (HDC-, H1R-, H2R-KO) and wild-type mice. Preliminary data derived from long-term recordings of synaptic activity and application of context- and behavioral state-dependent stimuli to two separate neuronal inputs in hippocampal slices in KO and wild-type mice suggest a role of histamine in specific macromolecular forms of synaptic plasticity (protein synthesis- and cytoskeleton-dependent L-LTP/LTD and synaptic tagging). Supplemented by analysis of the neurogenic capacity in KO and wild-type mice, these data reveal cellular and molecular mechanisms underlying histamine- and behavioral state-dependent control of learning and memory function

O 19

Expression of Histamine Receptors and Effect of Histamine in the Rat Carotid Body Chemoafferent Pathway

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Withdrawn

Oligomerization of the Human Histamine H_4 Receptor

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Over the last years the principal of GPCRs forming oligomers has become widely accepted. Although the use of biophysical approaches like FRET and BRET have enabled the detection and visualization of receptor oligomerization in living cells, still much of the oligomerization process and function is not fully understood.

The human histamine H_4 receptor (hH_4R) is the fourth and most recently discovered member of the histamine family of G protein-coupled receptors (GPCRs), and shares highest homology with the histamine H_3 receptor (H_3R). The hH_4R is thought to play a role in inflammation, and links to allergic rhinitis and colitis have recently been made, making this receptor a potential new target for drug intervention. Like the H_3R, the H_4R gene consists of several introns and exons, potentially resulting in differentially spliced isoforms, as have been detected for the H_3R. Although structurally similar, the expression patterns of both receptors hardly overlap; while the H_3R is expressed almost exclusively in the brain, the H_4R is expressed predominantly in hematopoietic cells. In terms of distribution and suggested physiological roles, the H_4R thus appears more closely related to the H_1R.

Here, using a specific polyclonal anti-hH_4R antibody and both biochemical (column chromatography) and biophysical approaches (BRET, FRET), we have studied the homo-oligomerization of the hH_4R. We have studied the oligomeric potential of the hH_4R to form hetero-oligomers with the hH_1R as well as with two splice variants of the hH_4R, we have recently identified. We found that the hH_4R constitutively forms homo-oligomers, which are present at the cell surface and that oligomer formation is not modulated by ligand stimulation. At physiological expression levels we failed to detect H_1R-H_4R hetero-oligomers. We detected hetero-oligomers between the H_4R and either of the H_4R splice variants, which resulted in a dominant negative effect on H_4R ligand binding. The hetero-oligomerization between the H_4R and a truncated H_4R splice variant suggest the involvement of transmembrane domain I-II of the H_4R in H_4R oligomerization.

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O 21

Homology Modeling of the Human Histamine H_4 Receptor and its Application in Structure-Based Drug Design

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The histamine H4 receptor (H4R) is the novel member of the histamine receptor family. Experimental results show, that it has a major role in calcium mediated chemotaxis on eosinophils and mast cells. It also affects IL-16 secretion from $CD8^+$ T cells. These results indicate a role in inflammation and allergy, therefore the H4R seems to be an interesting target for drug design.

Three dimensional model of the human histamine H4 receptor (HH4R) was developed by means of homology modeling. Six models were build by Modeller 6.2. The quality of the models were tested by validation softwares (Procheck, Whatcheck), and available mutational data from the literature.

The binding site of histamine was mapped, and several interaction points of histamine were explored. Histamine and other known H4 agonists and antagonists were docked successfully into the homology model with FlexX.

To evaluate whether our model is suitable for selecting HH4R active compounds from a dataset containing known actives and inactives, enrichment studies were carried out with diverse and druglike sets of molecules. We used FlexX in combination with the C-Score package, and were able to get enrichment factors of 50-60 with the most appropriate scoring function combination, which is very significant comparing these results with other enrichment studies on homology models. Therefore we decided to screen almost 8 millions of compounds in silico on our HH4R model. The best scored compounds from the virtual high throughput screening, will be also tested in vitro.

Characterization of 4-Methylhistamine and VUF 8430 as Selective Histamine H_4R Agonists

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Several lines of evidence have shown that the histamine H_4 receptor (H_4R) is an important player in inflammation processes, and this receptor has therefore been considered as a potential drug target for the treatment of some inflammation-related diseases (Fung-Leung *et al*., *Curr Opin. in Invest. Drugs* 2004 5:1174-1183). Thus, to further pharmacologically study the H_4R, selective and potent agonists and antagonists for this receptor are demanded. In our search for H_4R ligands, employing SK-N-MC cell line heterologously expressing the human H_4R, we have identified two selective H_4R agonists: 4-methylhistamine and VUF 8430. Both ligands show selectivity for H_4R over other histamine receptor substypes, and they display full agonistic activity at human, rat, and mouse H_4Rs. 4-Methylhistamine was further investigated in *ex vivo* experiments, and it dose-dependently induces migration of bone marrow-derived murine mast cells and human eosinophils. In conclusion, we have discovered two compounds that may potentially serve as H_4R pharmacological tools.

O 23

Histaminergic System Shapes Network Synchonization in the Hippocampus and Striatum in the Behaving Rat

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It is thought that aminergic systems in the brain coordinate excitability of multiple neuronal classes to assist in fitting neural network performance to adaptive demands. A form of temporal organization of the neuronal activity is its periodic synchronization manifested as oscillations of the local field potential at various frequencies. An unresolved problem is how network oscillations are coordinated in time and frequency domains by neuromodulatory systems. The electrophysiological effects of systemic injections (i.p.) of pyrilamine, an H1-receptor antagonist, zolantidine, an H2-receptor antagonist, and ciproxifan, an H3-receptor antagonist, were measured in the behaving rat (n=6). The occurrence of ripple (~200 Hz) oscillations in the hippocampus was strongly facilitated by pyrilamine (5 mg/kg, to 184% of baseline) and was transiently reduced by zolantidine (10 mg/kg, to 54% of baseline). Thioperamide (5 mg/kg) did not affect ripple oscillations. Histaminergic modulation of theta, gamma and ripple oscillations in the hippocampus (ventral CA1 area) and slower oscillations in the striatum as well as their interaction were further revealed by conventional cross-spectral coherence methods and non-linear generalized synchronization analysis with a reference to vigilance states of an animal.

Enhanced CREB Activity and Adipogenic Potential in Histamine Free Embryonic Fibroblasts

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Histamine has been studied for 100 years, but little is known about the importance of its deficiency. Histidine-decarboxylase gene-targeted (HDC KO) mice provide a model to study how the lack of histamine impacts physiological and cellular mechanisms. CREB, a key transcriptional factor in adipogenesis can be induced by histamine though different pathways. The goal of this work was to study histamine signalling with a special focus on CREB as well as the adipogenic potential of HDC KO cells in the absence of histamine. We measured CREB activity and expression by EMSA, Western blot and real-time RT-PCR, as well as cAMP levels by ELISA in primary embryonic fibroblasts derived from WT and HDC KO mice. The ability of these cells to form adipocytes was also tested using a low-potent induction cocktail of IBMX, insulin and dexamethasone applied according to the standard differentiation protocol. Differentiation process was monitored by phase contrast microscope and verified by Oil Red O staining. H1, H2 and H3 receptor expression was measured by real-time RT-PCR. As a result we found that in contrast to wild type cells, HDC KO fibroblasts show enhanced constitutive CREB activity and nuclear localisation accompanied by a 4-fold increase in intracellular cAMP levels. Along with these findings HDC KO cells appeared to be more potent to differentiate into adipocytes. There was no difference in the expression of histamine receptors, excluding the possibility that high cAMP level is due to receptor overexpression. In this study we showed that chronic histamine deficiency directly impacts the formation of adipocytes, likely through modifying CREB-related transcriptional events mediated by second messenger cAMP. These data contribute to uncover histamine-regulated transcriptional networks and introduce a new aspect in elucidating the complex role of histamine in energy homeostasis.

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Excitation of Aminergic Neurons by Histamine

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Mutual regulation of aminergic systems is crucial for the adaptive control of brain functions. The histaminergic system is our major waking center. Its neurons send dense projections through the entire brain and excite cholinergic, serotonergic and dopaminergic neurons. Only the noradrenergic locus coeruleus (LC) has not previously been examined. We report here the effect of bath-applied histamine on cells in the LC by single-unit recordings in slices and the expression of histamine receptors in this area by single-cell RT-PCR. We compare this action to that on the other aminergic nuclei. Histamine (10 μM) increased the firing of NAergic cells to 130 ± 9 % of control, 100 μM to 256 ± 58% of control. This excitation was unaffected by blocking synaptic transmission. Histamine-mediated excitation was blocked by a H_1 receptor antagonist, mepyramine, in 78% of cells and by cimetidine, a H_2 receptor antagonist, in 42% of cells, but not by the H_3 receptor antagonist thioperamide. RT-PCR revealed that mRNA for the H_1 receptor was expressed in 77% of isolated LC neurons, mRNA for the H_2 receptor in 41% of LC neurons and H_3 receptors in 29%. These findings underline the coordination between aminergic systems and suggest that the arousal induced by the histamine system involves excitation of noradrenergic neurons in the locus coeruleus, cholinergic neurons in the nucleus basalis / septum, serotonergic neurons in the raphe and dopaminergic neurons in the substantia nigra and the ventral tegmental area.

Biochemical and Pharmacological Evidence for Homo- and Heter-Oligomeric Human H_3 Receptor Isoforms: Further Evidence of Dominant Negative Role for Splice Isoforms

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The histamine H_3 receptor (H_3R), expressed almost exclusively within the CNS, is a classic G-protein coupled receptor (GPCR). Alternative splicing of the H_3R gene gives rise to several isoforms, both in the rat and human brain. The various isoforms are distinct in their CNS distribution (with many areas of overlapping expression), and agonist pharmacologies, suggesting the possibility of hetero-oligomerisation. In this study, we report the homo- and hetero-oligomerization of two common human H_3R isoforms (445 and 329) by biochemical (Western blot), biophysical (*tr*-FRET) and pharmacological techniques.

To achieve this, we have generated the first anti-hH_3 445 isoform-specific antibody (Shenton *et al,* 2005; Bakker *et al*., 2006). Using our anti-hH_3R antibodies (Chazot *et al*., 2001), we have demonstrated the presence of robust H_3R oligomers in both HEK 293 cells heterologously overexpressing H_3Rs, as well as in human putamen endogenously expressing H_3Rs.

Here, we show using *tr*-FRET and immunoblotting techniques that both the hH_3 445 and hH_3 329 isoforms readily form robust homo-oligomers. Immunoblotting data provide evidence that the hH_3 329 isoform displays a greater propensity to homo-oligomerise than the hH_3 445 isoform. Furthermore, using *tr*-FRET technique, we provide the first evidence that these two human isoforms can form homo- and hetero-oligomers. Previously, we have shown that the rH_{3D-F} isoforms can modulate the cell surface expression and functionality of the rH_{3A} isoform, ie act as dominant negative isoforms (Van Rijn *et al*., 2006). Based on preliminary saturation binding assays using [^{3}H] clobenpropit, we show that the presence of the hH_3 329 isoform has a dominant negative effect upon the binding affinity of [^{3}H] clobenpropit for the hH_3 445 isoform. This suggests the distinct possibility that alternative splicing in conjunction with oligomerisation have profound roles in regulating rodent and human H_3 receptor pharmacology and functionality *in vivo.*

Chazot PL, Hann V, Wilson C, Lees G, Thompson CL. NeuroReport 2001;12:259-262

Bakker RA, Lozada AF, van Marle A, Shenton FC, Drutel G, Karlstedt K, Hoffmann M, Lintunen M, Yamamoto Y, Van Rijn RM, Chazot PL, Panula P, Leurs R. Mol Pharmacol. 2006 (in press)

Shenton FC, Hann V, Chazot PL. Inflammation Res. 2005;54:48-49

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O 27

New Signaling Pathways for the Histamine H_3 Receptor

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Drugs targeting the histamine H_3 receptor (H_3R) are suggested to be beneficial for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Leurs et al., 2005). The H_3R activates $G_{i/o}$-proteins to inhibit adenylyl cyclase (AC) activity and to modulate phospholipase A_2 (PLA_2) and MAPK. Herein we show that in transfected SK-N-MC cells, the human H_3R (445) also modulates the activity of the Akt/Glycogen synthase kinase 3β (GSK-3β) axis both in a constitutive and agonist dependent fashion. H_3R stimulation with the H_3R agonist immepip induces the phosphorylation of both Ser^{473} and Thr^{308} on Akt, a serine/threonine kinase that is important for neuronal development and function. The H_3R-mediated activation of Akt can be inhibited by the H_3R inverse agonist thioperamide, as well as by wortmannin and PTX, suggesting that Akt activation occurs via a $G_{i/o}$-mediated activation of phosphoinositide-3-kinase (PI(3)K). H_3R activation also results in the phosphorylation on Ser^9 of GSK-3β, which acts downstream of Akt and has a prominent role in brain function.

In addition, we show H_3R-mediated release of intracellular calcium, which can be inhibited by the H_3R inverse agonist thioperamide and PTX, suggesting that this occurs through $G_{i/o}$-proteins. Identification of these newly discovered signaling properties of the H_3R adds new understanding to roles of histamine and the H_3R in brain function and pathology.

Leurs R, Bakker RA, Timmerman H, de Esch IJ. Nat Rev Drug Discov 2005;4:107-120

Histamine H_3 Receptor Expression is Increased in the Brains of Transgenic Mice Over-Expressing Beta Amyloid

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Histamine H3 receptor antagonists have been shown to enhance learning and memory in a number of animal models. This is thought to be mediated through regulation of neurotransmitter levels, including histamine and acetylcholine. In an effort to determine the utility of H3 receptor antagonists for the treatment of cognitive deficits in Alzheimer's disease (AD), we have investigated the expression of H3 receptors in transgenic mice (Tg2576) over expressing the Swedish mutation of the human amyloid precursor protein which display biochemical, pathological, and behavioral markers consistent with many aspects of AD, including impaired hippocampal function. Using immunohistochemistry, in brain sections from 18 week old mice that have elevated beta amyloid (A beta) levels and cognitive deficits, we have shown that H3 receptor expression is increased in the cortex, hippocampus and cerebellum of these mice. The increase in H3 receptor immunostaining in hippocampus co-localized with neuronal markers and is concentrated in CA3 region. These results suggest that H3 receptor expression and possibly function is altered in the Tg2576 mice. These changes in H3 receptor expression by A beta in a mouse model of AD may provide a preclinical correlate of the previously reported decreases in histamine levels in hypothalamus, hippocampus and temporal cortex of brains from Alzheimer's disease patients (Panula et al., 1998) and further support the potential utility for H3 receptor antagonists for the treatment of AD.

Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H, Relja M. Neuroscience 1998;82:993-997

O 29

Different Response to GABAA or H_3 Antagonists Suggest the Existence of Distinct Subpopolations among Histaminegic Neurons

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Neuronal histamine (HA) is released from axon varicosities innervating the entire brain, and originating within the only source of HA fibers, the tuberomammillary nucleus (TMN). Several receptors control HA neurons activity. Using dual-probe microdialysis, we studied the effect of an H_3 antagonist/inverse agonist, thioperamide, and a GABAA antagonist, bicuculline, on HA release. Significant effects were determined by ANOVA /Fisher's test. SD rats were implanted with 2 probes, the 1st one always in the TMN, and the 2nd one in a projection area, the nucleus basalis magnocellularis (NBM), nucleus accumbens (NAc), dorsal striatum (DS) or prefrontal cortex (PFC). Freely-moving rats were perfused with Ringer at 2-µl/min rate, and 15-min samples were collected. HA was measured by HPLC-fluorometric detection (Yamatodani et al, J Chromatogr 1985, 344: 115). Spontaneous HA release from all regions was stable and ranging 0.05-0.08 pmol/15min (N=31). Thioperamide (300nM) or bicuculline (10 µM) were infused for 60 min into the TMN, where HA cell bodies are localized. Both drugs increased HA release from the TMN and PFC, but not from the DS. Thioperamide, but not bicuculline increased HA release from the NBM. Conversely, bicuculline and not thioperamide increased HA release from the NAc. These data show that GABA and HA tonically modulate HA neuronal activity. Another key finding is the heterogeneous response elicited by intra-TMN administration of either thioperamide or bicuculline on HA release from distinct projection areas, thus suggesting the presence of functionally distinct HA neuronal populations. Consistently, it has been reported that HA neurons give different responses to distinct stress stimuli (Miklos et al, Eur J Neurosci 2003, 13: 3069). Drugs acting on H_3 receptors can have an important role in the treatment of sleep disorders, decline of memory and obesity. Acting only on specific subpopulation may achieve more selective effects, and reduce the collateral effects.

Cloning and Expression of Histamine H_1, H_2 and H_3 Receptors in Zebrafish and Effects of their Ligands on Behavior

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Methods to analyze plastic changes in neurotransmitter networks and quantify behavior of a large number (up to 100) of zebrafish simultaneously were developed. This opens possibilities to study the effects of gene modification on complex neural systems, and to test drugs for CNS diseases in a vertebrate model. Histamine H_1, H_2 and H_3 receptors were cloned from the zebrafish brain. The zebrafish H_1 gene was located on chromosome 8. The intronless gene codes for a 458 aa long protein. The zebrafish H_2 receptor on chromosome 15 was an intronless gene, which displays 60% identity with the corresponding sequences from dog, human, rat and mouse. The zebrafish H_3 receptor gene located on chromosome 7 contained four exons. The first exon contains the start site and continues to the middle of TM2, the second exon runs to the beginning of IC2. The third exon ends in the middle of IC 3 and the final exon contains the rest of the coding sequence. Sequence analysis revealed 52-64% identities with the corresponding sequences from rat and human depending on which isoforms are used for comparison. The 1158 bp long coding sequence shows highest identities to rat H_{3C} and human isoform H_3 5. The three receptors were expressed in the brain and several other tissues.

Automated behavioral analysis of adult zebrafish after ligand exposure through water (50 uM) revealed increased locomotor activity after tiotidine, thioperamide and clobenpropit. Altered swimming behavior due to changes in anxiety-related behavior was observed after thioperamide and clobenpropit. The zebrafish histamine receptors resemble those of higher vertebrates and they provide a useful model for pharmacological and behavioral studies for characterizing the functions of histamine in detail. Exposure of zebrafish embryos to some histamine H_1, H_2 or H_3 ligands for 24-72 h induced developmental abnormalities, which suggests that histamine receptors may also be involved in developmental processes.

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A New Family of Histamine H_3 Receptor Antagonists Based on a Natural Product: Discovery, Development of SAR, and Properties of the Series

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A new family of histamine H3 receptor antagonists based on a plant-derived alkaloid (conessine) has been discovered. This series is unique in having no aromatic or heteroaromatic rings. Although moderately potent at histamine H3 receptors (Ki human H3R 5 nM, rat H3R 12.3 nM) natural compound itself had unacceptable properties overall to allow further development, so SAR studies to address key areas were carried by synthesizing new series inspired by conessine. A structural model was formulated for the pharmacophore, based on activity of specific synthetic compounds, and also by inferred homology to general H3 receptor antagonist pharmacophores. New compounds were designed with high potency across target species' H3 receptors (Ki as low as 0.2 nM in human H3R, 0.5 nM in rat H3R), and have good in vivo, PK (65% oral bioavailability in rat, brain/plasma ratios >11x), and physicochemical properties. All compounds tested were antagonists of histamine H3 receptors, and further tests indicated that they act as inverse agonists. The efficiency of CNS penetration will be discussed, as well as selectivity of the compounds in comparison to reference standard antagonists.

Histamine H_3 Antagonists with Serotonin Reuptake Transporter Inhibitor Activity

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Depression is a major health issue, with more than 340 million people being affected worldwide. Many patients who suffer from depressive disorders are also diagnosed with cognitive impairment and fatigue. Depression is often treated with one of the currently marketed selective serotonin reuptake inhibitors (SSRIs), however these agents often fail to improve the cognitive impairment and fatigue observed with many patients even as mood improves. In fact, some SSRIs even induce fatigue and excessive sleepiness. One strategy used to improve the efficacy of SSRIs has been to co-administer modafinil. Although the mechanism of action of modafinil is unknown, the compound has been shown to improve cognition and increase wakefulness. Similar effects have been observed in pre-clinical studies with histamine H_3 antagonists. These studies have demonstrated the histamine H_3 antagonists have pro-cognitive effects and show increased wakefulness without inducing nonspecific stimulant effects. This information led us to investigate the utility of histamine H_3 antagonists with serotonin reuptake transporter inhibitor activity. Towards this end, we have discovered a series of isoquinolines that are potent histamine H_3 antagonists and serotonin reuptake inhibitors. The compounds readily penetrate the CNS and several members of this series have potent in vivo activity. The medicinal chemistry, in vitro data, and in vivo pharmacology of this novel series of dual acting compounds will be presented.

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Pharmacological Classification of Histamine H_3 Receptor Agents across Species is Attributable to TM3 Sequence Differences

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Despite high sequence homology between rat and human histamine H_3 receptors, large affinity differences between species have been observed for a range of compounds in radioligand binding assays. Two residues within TM3 have been implicated and are hypothesized to play a critical role in ligand recognition. To investigate the H_3 receptor functional pharmacology across species, H_3 ligands were tested for modulation of [^{35}S]GTPγS binding. Significant species-dependent differences in potency and efficacy were determined with some compounds displaying differential functional activity dependent upon the species assayed. Imoproxyfan, imhexamine, FUB 322, and VUF5681 were agonists in stimulating [^{35}S]GTPγS binding at the human H_3 receptor, but were inverse agonists, inhibiting basal [^{35}S]GTPγS binding, at the rat H_3 receptor. Iodophenpropit and cyclopentyl-impentamine did not alter basal [^{35}S]GTPγS binding at the human H_3 receptor (neutral antagonism) but were inverse agonists at the rat H_3 receptor. Human H_3-like pharmacology was observed with a chimeric H_3 receptor (composed of amino acids 1-144 of the human and 145-445 of the rat H_3 receptor). However, when residues T119 and A122 within TM3 of the human H_3 receptor were mutated to their corresponding residues in the rat receptor (A and V, respectively), the pharmacological profile was essentially the same as that seen with the rat H_3 receptor. This pharmacological heterogeneity did not appear to be caused by differing levels of constitutive H_3 receptor activity in these cell lines. Together, these data demonstrate that significant differences in ligand pharmacological classification exist across species for the H_3 receptor, a result that has important implications in drug discovery. Further, the demonstration that two residues within TM3 are responsible for this effect implies that these amino acids are important in determining the species-specific conformation of H_3 receptors

Funded by Abbott Laboratories.

Fluorophore-Tagged Non-Imidazole Histamine H_3 Receptor Ligands with Subnanomolar Affinities

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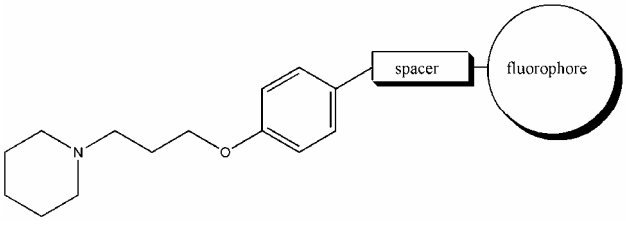
Fluorescent small molecules as bioactive ligands for G protein coupled receptors have rarely been shown because normally the large and bulky fluorescent moieties decrease affinities. Some examples for histamine H_1, H_2 and most recently H_3 receptors have already been described [1,2]. Based on recent findings on structure-activity relationships on non-imidazole histamine H_3 receptor antagonists a 1-(3-phenoxypropyl)piperidine pharmacophore has been developed which can be substituted in *para*-position of the aromatic ring by different bulky groups maintaining or even increasing affinity. Primary amino or alcohol functionalities were mostly used as linking groups. The pharmacological assay is based on [^{125}I]iodoproxyfan displacement on CHO-K1 cells stably expressing the hH_3 receptor.



Within numerous novel potent histamine H_3 receptor ligands with different fluorescent moieties we found simple derivatives of Sanger's reagent as highly interesting compounds (spacer; NH, O, CH_2-NH, and phenyl-CH_2-NH having K_i values of 0.14 nM, 0.048 nM, 0.603 nM, and 0.706 nM, respectively). The compounds ranged by CLogP values for lipophilicity from 5.0 to 7.0, by excitation wave lengths from 396 to 452 nm, and by emission wave lengths from 449 to 531 nm. Stoke shifts varied from 53 to 130 nm. The fluorescent compounds showed affinities in the picomolar concentration range and are promising ligands for further in vitro and in vivo investigation.

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[2] Cowart M. et al. 34th EHRS Meeting, Bled/Slovenia (May 11-14, 2005), Abstract book p. 49.

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The Histamine H_4 Receptor Mediates Allergic Airway Inflammation

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Histamine is an important inflammatory mediator that is released in airways during an asthmatic response. However, current antihistamine drugs are not effective in controlling the disease. The discovery of the histamine H_4 receptor (H_4R) prompted us to reinvestigate the role of histamine in pulmonary allergic responses. H_4R deficient mice and mice treated with H_4R antagonists exhibited decreased allergic lung inflammation in a model of asthma, with decreases in infiltrating lung eosinophils and lymphocytes and decreases in T_H2 responses. Ex vivo restimulation of T cells showed decreases in cytokine production and treatment with a H_4R antagonist leads to improvements in airway hyperresponsiveness in response to methacholine, suggestive that T cell functions are disrupted. In vitro studies indicated that blockade of the H_4R on dendritic cells leads to decreases in cytokine and chemokine production and limits their ability to induce T_H2 responses in T cells. This work suggests that the H_4R can modulate allergic responses via its influence on T cell activation. The study expands the known influences of histamine on the immune system and highlights the therapeutic potential of H_4R antagonists in allergic conditions.

Distribution Pattern of Histamine H_4 Receptor in Human Synovial Tissue from Patients with Rheumatoid Arthritis

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Rheumatoid arthritis (RA), a chronic inflammatory disease, is characterized mainly by immunological disturbances in synovial tissue, but its pathogenesis remains unclear.

Very little is known about the biological function of the novel histamine H4 receptor (H4R) and the recent experiments in vivo indicate its role in inflammatory conditions and immune system disturbances. There is also an evidence that H4 receptor plays a key role in chemotaxis of eosinophils and mast cells to amplify histamine-mediated allergic reactions. Expression of H4R has been observed in eosinophils, T cells, dendritic cells, basophils, and mast cells in blood and many organs.

While the presence of H4 receptor in cultured synovial cells of RA patients has been previously documented (*Ikawa Y. Biol Pharm Bull 2005*), distribution pattern of this receptor in synovial tissue slides has not been determined as yet.

Synovium samples were obtained at joint replacement surgery of RA patients or from young adults as control. Samples were fixed in 4% paraformaldehyde and H4R was localized by immunohistochemical staining using the primary polyclonal antibodies.

RT-PCR technique confirmed the H4R specific mRNA expression in examined specimens. We demonstrated the presence of H4 protein in various types of synovium cells. Histamine receptor H4 was found in the synoviocytes of the superficial layer membrane of synovium and synovial villi. H4R was also localized in cells of vascular walls and inflammatory cells infiltrated perivascular area.

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

O 37

Theophylline as "Add-on" Therapy to Cetirizine in Patients with Chronic Idiopathic Urticaria: a Randomized, Double-Blind, Placebo-Controlled Pilot Study

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Chronic urticaria is a condition associated with itching and substantial disability, but its pathogenesis is not well understood. Patients could have either chronic autoimmune or chronic idiopathic urticaria (CIU). Treatment is mostly limited to the use of antihistamines.

To investigate if the non-specific phosphodiesterase inhibitor theophylline could provide additional benefit to the histamine-1 receptor (H-1R) antagonist cetirizine.

This was a double-blind, randomized, placebo-controlled, parallel study. Patients with CIU, with negative autologous serum skin test, were randomized to two treatment groups: Group A with 67 subjects in combination therapy of cetirizine (10 mg) + theophylline (200 mg) in the morning and theophylline (200 mg) at night; Group B with 67 subjects in monotherapy of cetirizine (10 mg) with placebo in the morning and placebo at night for 6 months. Group A patients took theophylline only in the morning for additional 6 months. The entire study was completed by 54/67 (80.6%) subjects in group A and 51/67 (76.1%) in group B. Patient response was assessed by a visual analog scale (VAS) and a treatment effectiveness score (TES). Blood theophylline levels were also determined at the first and seventh months of treatment.

The addition of theophylline provided considerable benefit that was statistically significant from the second month on, regardless of the person performing the evaluation (patient or physician), both in the VAS and the TES results. A direct inhibitory effect of up to 2 hour pretreatment with theophylline could not be demonstrated on cultured human mast cells.

Addition of theophylline to conventional H-1R antagonists provides considerable benefit in the management of CIU that may require 1-2 months before it is evident.

Theoharides TC, Cochrane D. Critical role of mast cells in inflammatory diseases and the effect of acute stress. J Neuroimmnunol 2004;146:1-12

Biphasic Immunomodulating Effect of Cepharolsporin Derivatives in Man *in Vitro* and *in Vivo*

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In our earlier studies on the immunological cross-reactivity between penicillins and cephalosporins (CFS), we had noted that high concentration of cephaloridine (> 10 μM) inhibited lymphocyte proliferation, while low concentrations tended to enhance it [1]. Later it was reported that CFS and other antibacterial agents have immune modulating properties [2, 3].

In the current study, we have further investigated the effect of a selection of CFS on the uptake of 3H-thyrmidine by human peripheral blood lymphocytes, stimulated with antigen, tuberculin PPD or phytohaemagglutinin (PHA) (cepfalexin, cephalothin and cephaloridine, 1st generation CFS and its conjugate with bovine γ-globulin (BGG); cefuroxime, 2nd generation CFS; ceftazidime and cefotaxime, 3rd CFS). Penicillins have no such effects.

Generally (with some major differences between different CFS), lymphocyte responses to antigen, PPD and PHA were potentiated (by up to 120%) in the presence of low concentrations of CSF (0.1-20 μM in the culture medium) and inhibited by high concentrations (50-200 μM). For instance, ceftazidime at 10 μM caused 50% increase ($P < 0.02$), while at 50 μM and above it induced 80% reduction ($P < 0.001$). Cephaloridine caused similar effects, and it was interesting to find that its BGG conjugate (covalent) was also active, but predominantly inhibitory (by up to 74%).

To assess the significance of CFS effects on lymphocytes, we tested the effect of cephaloridine and its covalent conjugate with human serum albumin (HAS) in the tuberculin skin test with purified protein derivative (PPD), which elicits a classical delayed-type hypersensitivity reaction, in human subjects. High concentrations of cephaloridine (50 μM or above) or its HSA conjugate inhibited the skin response, while low concentrations potentiated it.

IgE-dependent histamine release from human basophils was less consistently modulated by cephaloridine and its BGG conjugate, showing potentiation or inhibition.

These results show that CFS and their protein conjugates have significant biphasic in vitro and in vivo immunomodulatory effects on delayed-type, but not immediate-type, hypersensitivity reactions.

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O 39

On the Mechanism of Antiphagocyte-Antioxidative Effect of H_1-Antihistamines

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In addition to their antihistamine effects, H_1-receptor antagonists possess other pharmacological properties which are not uniformly distributed among drugs of this class, such as anti-inflammatory effects, inhibition of blood platelet functions and antioxidant effects

The effect of histamine (HIS) and two H1-antihistamines, dithiaden (DIT) and loratadine (LOR) was investigated on chemiluminescence (CL) of human whole blood (WB), isolated poyzmorphonuclear leukocytes PMNL and on the production of superoxide (SO) and myeloperoxidase (MPO) generation after stimulation with phorbol-myristate acetate (PMA).

Blood collection and PMNL separation were processed by differential centrifugation of fresh anticoagulated human blood as described earlier. Luminol-enhanced CL was detected by means of luminol and isoluminol in Luminometer LM-01T[6]. For determination of SO and MPO the respective enzymatic and CL-method was applied, respectively.

HIS did not affect CL of WB, but dose-dependently decreased CL and liberation of MPO from isolated PMNL. DIT and LOR decreased CL of WB and PMNL in a concentration dependent manner and decreased production of SO and MPO release from isolated PMNL. Since HIS does not pass intracellularly, its effect on isolated PMNL may appear on two levels. First, it interferes with CL extracellularly (scavenging effect), second, it might interact with the generation of free oxygen radicals through PMNL membrane H1 receptors.

Representatives of the first and second generation of H1 antihistamines DIT and LOR proportionally decreased both the extra- and intracellular part of CL in PMA-stimulated PMNL. Since PMA stimulated PMNL bypassing membrane receptors it is suggested that both H1 antihistamines interact with CL at intra- as well as extracellular sites. This indicates that the H1-antihistamines investigated suppressed the formation of free oxygen radicals (intracellular part of inhibition) as well as scavenged free radicals outside PMNL.

The suppressive effect of H1-antihistamines on phagocytosis and free radical generation is indicative of the beneficial side effect of these drugs used in inflammatory processes. On the other hand, due to their suppressive effect on phagocyosis of PMNL, this effect must be considered carefully when H1-antihistamines are used during infection.

Evidence of Human Mast Cell Function in Inflammatory Diseases

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This presentation consists of the following 3 abstracts

Regulation of Histamine Secretion from Human Mast Cells

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Histamine secretion is typically associated with mast cell activation. However, mast cells can release other vasoactive and inflammatory molecules without histamine. thus explaining the involvement of mast cells in inflammatory conditions. A number of natural endogenous and exogenous molecules have been shown to inhibit histamine secretion, but they have not been adequately studied. As a result, there has been a paucity of clinically effective mast cell blockers.

To review evidence for natural innate or exogenous molecules that could regulate histamine secretion.

Publications in Medline and unpublished experimental evidence was compiled.

Heparin, chondroitin sulfate, histamine-3 receptor agonists, nitric oxide, retinoic acid and spermine are innate molecules, while quercetin and kaempferol are plant-derived flavonols, which can inhibit histamine secretion. However, some of these molecules can also inhibit the synthesis and release of distinct cytokines. Moreover, IL-1 can stimulate selective release of IL-6, while CRH can induce selective VEGF release without histamine through distinct pathways or inhibition of histamine secretion.

These results indicate that a number of natural inhibitors of histamine secretion could be exploited as potential anti-allergic and anti-inflammatory drugs.

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Human Mast Cells Express Functional Corticotropin-Releasing Hormone Receptors (CRH-R) Leading to Selective Secretion of Vascular Endothelial Growth Factor (VEGF)

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Mast cells are critical for allergic, but also for inflammatory reactions that worsen by stress. CRH activates the hypothalamic-pituitary-adrenal axis under stress, but has proinflammatory peripheral effects through mast cells.

We investigated the expression of CRH-R and the effects of CRH in human mast cells.

Human umbilical cord blood-derived mast cells (hCBMCs) were evaluated with RT-PCR and were stimulated with CRH, following which cAMP, histamine, IL-8, TNF-α and tryptase were measured.

We detected mRNA for CRH-R1a , 1b, 1c, 1e, 1f isoforms and CRH-R1 protein. CRH-R2α (but not β or γ) were also present. CRH increased cAMP and induced secretion of VEGF without tryptase, histamine, IL-8 or TNF-α. These effects were blocked by the CRH-R1 antagonist Antalarmin, but not the CRH-R2 antagonist Astressin 2B. CRH-stimulated VEGF production was mediated through increased cAMP since the effect of CRH was mimicked by the direct adenylate cyclase (AC) activator forskolin and the cell-permeable cAMP analogue 8-bromo-cAMP, while it was abolished by the AC inhibitor SQ22536.

CRH-induced mast cell VEGF could be involved in inflammatory conditions, such as arthritis or psoriasis, and CRH-R antagonists could have therapeutic potential.

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Myelin Basic Protein (MBP) Induces Selective Release of IL-8 without Histamine from Human Mast Cells

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CD4+ TH1 cells mediate demyelination in multiple sclerosis (MS), but how they get sensitized and enter the brain to induce inflammation remains obscure. TH2 cytokines associated with allergic disorders have recently been implicated in MS, while genes upregulated in MS plaques include mast cell-related tryptase, IgE receptor (FcepsilonRI) and histamine-1 receptor. Brain mast cell activation increases blood-brain-barrier (BBB) permeability that precedes any clinical or pathologic signs of MS.

Investigate the ability of MBP to stimulate human mast cell secretion of inflammatory mediators.

Human umbilical cord blood-derived mast cells (hCBMCs) were stimulated with human MBP or with anti-IgE as positive control, following which histamine, IL-8 and tryptase were measured.

MBP (10 mcM) increased histamine and tryptase release from about 18% in the control to 40%, as compared to 80% with anti-IgE. IL-8 was increased from 160 pg/10^6 cells in the control to 4185 with MBP and 16766 with anti-IgE.

These results indicate that MBP can activate mast cells to release pro-inflammatory molecules that could disrupt BBB and permit T-cell sensitization. Inhibiting brain mast cell activation may be a novel therapeutic target for MS.

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The Possible Role of Mast Cells and VEGF in Peritumoral Oedema of Secretory Meningioma

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Withdrawn

Skin Histidine Decarboxylase (HDC) and Corticotropin-Releasing Hormone Receptor-1 (CRH-R1) Genes Are Overexpressed in Chronic Urticaria (CU)

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Certain skin disorders, such as contact dermatitis and CU, are characterized by inflammation involving mast cells and worsen by stress. However, the underlying mechanism of this effect is not known. The skin appears to have the equivalent of a hypothalamic-pituitary-adrenal (HPA) axis, including local expression of (CRH and its receptors. We have reported that acute stress and intradermal administration of CRH stimulate skin mast cells and increase vascular permeability through CRH-R1 activation.

We investigated the expression of CRH-R1, the main CRH-R subtype in human skin, and the mast cell related gene HDC, which regulates the production of histamine, in normal and pathological human skin biopsies.

Quantitative real time PCR was used on skin biopsies frozen immediately in RNA preservation solution.

Results: CU expressed high levels of CRH-R1 and HDC as compared to normal foreskin, breast skin and cultured human keratinocytes.

These results implicate CRH-R in CU, which is often exacerbated by stress, and indicate that CRH-R1 antagonists may be added to histamine-1 receptor antagonists for possible synergistic therapeutic effect.

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O 43

Role of Histamine in Ghrelin-Induced Gastroprotection against Acute Gastric Lesions

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Histamine and agonists of histamine H1 and H2 receptors were shown to afford gastroprotection against gastric mucisal damage, but whether histamine is implicated in gastroprotective action of ghrelin, an orexigenic peptide of gastric mucosa origin, remains unknown.

We studied the effect of ghrelin applied intraperitoneally (i.p.) in a graded doses ranging from 2.5-40 μg/kg on gastric secretion in conscious rats equipped with gastric fistula (GF) and gastric mucosal lesions induced by 100% ethanol with or without: 1) inhibition of H2 receptors by ranitidine (30 mg/kg i.g.) and H3 receptors by clobenpropit (1μM/kg i.p.), 2) suppression of histidine decarboxylase (HDC) activity with alpha-fluoromethyl histidine (alpha-FMH). The area of gastric lesions was measured by planimetry, the gastric blood flow (GBF) was determined by H2-gas clearance technique and blood was withdrawn for the measurement of plasma ghrelin and histamine levels by specific radioimmunoassays (RIA). The expression of HDCmRNA and ghrelin was analyzed by RT-PCR.

Ghrelin dose-dependently increased gastric acid output in GF rats and this effect was completely abolished by ranitidine and significantly attenauted by alpha-FMH. Ethanol produced widespread mucosal lesions and this was accompanied by the rise in gastric mucosal blood flow and gastric mucosal expression of ghrelin mRNA. Exogenous ghrelin dose-dependently attenuated ethanol-induced gastric lesions and this effect was accompanied by a significant upregulation of HDC mRNA. The protective effect of ghrelin was significantly attenuated by ranitidine and α-FMH, but not by clobenpropit pretreatment.

Ghrelin exerts a potent gastroprotective action against acute gastric mucosal injury induced by noxious agent such as ethanol due to upregulation of HDC mRNA resulting in an excessive histamine release

Gastric Effects of the Histamine H_4 Receptor Antagonists JNJ7777120 and VUF6002

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The recently cloned histamine H_4 receptor is mainly expressed in periphery and, in particular, in haematopoietic and immune cells and a role in inflammation has been proposed [1]. Preliminary data [2] have shown that the selective H_4 receptor antagonist JNJ7777120 significantly reduced gastric lesions induced by indomethacin. In the present study, the role of histamine H_4 receptors on gastric acid secretion and gastric mucosal defence was investigated in rats by using JNJ7777120 and its benzimidazole derivative VUF6002, which in binding studies was characterised as a potent and selective H_4 receptor antagonist [3]. In anaesthetised rats with lumen-perfused stomach, JNJ7777120 (3-10 mg/kg iv) and VUF6002 (10-30 mg/kg iv) did not modify basal acid secretion; JNJ7777120 (3-10 mg/kg iv) induced a dose dependent-reduction of the acid response to pentagastrin (10 µg/kg iv), while leaving unaltered the hypersecretion induced by histamine (40 mg/kg/h). VUF6002 (30 mg/kg iv) did not modify pentagastrin-induced acid output. JNJ7777120 (10-30 mg/kg sc) significantly reduced the gastric lesions produced by indomethacin (20 mg/kg sc) and by the mast cell degranulator compound 48/80 (0.75 mg/kg ip), maximal reductions being 70% and 77%, respectively. By contrast, JNJ7777120 (10 mg/kg sc) did not modify the massive haemorrhagic ulcerations induced by the necrotizing agent HCl 0.6N ig. VUF6002 (30 mg/kg sc), but not its chemical control VUF6007 (30 mg/kg sc), significantly reduced gastric damage induced by indomethacin (maximal reduction 80%). In conclusion, these data suggest a role for histamine H_4 receptors in gastric mucosal defence against acid-dependent ulcerogenic stimuli.

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O 45

G Protein Receptor Kinases (GRKs) Regulate Histamine H_2R Desensitization in Human Leukemic Cells

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Activation of H2R triggers adenylyl cyclase stimulation leading to cAMP accumulation and PKA activation. We have previously reported that GRKs participate in the regulation of H2R. Thus, in COS-7 cells cotransfected with H2R and GRKs we demonstrated that H2R rapid desensitization involves receptor phosphorylation by GRK2 and GRK3 (Mol. Pharmacol., 60:1049, 2001). Further in U937 promonocytic cell line endogenously expressing H2R as well as GRK2, GRK3 and GRK6, we showed the role of GRK2 in H2R desensitization (Mol. Pharmacol., 62:1506, 2002). In the present study we sought to establish the participation of GRK3 and GRK6 in H2R desensitization in U937 cells. For this purpose clones expressing low levels of GRK3 and GRK6 were generated by stable transfection with cDNA antisense for these kinases. Cyclic AMP was assayed following dose response curves as well as kinetic and desensitization experiments performed in the presence of a selective H2R agonist. Anti GRK3 clones showed a maximal response that resulted 25% to 47% lower than that of U937 cells. Further time course studies showed that these clones evoked reduced cAMP levels as compared to U937 cells and suffered a more rapid desensitization. Binding assays revealed that this behavior did not result from a reduced H2R number of sites in GRK3 clones. The response of anti-GRK6 clones was similar to that of U937 cells supporting that GRK6 is not involved in H2R desensitization. In view of the unexpected behavior shown by anti-GRK3 clones, GRK2 levels were determined by Western Blot. In anti-GRK6 clones GRK2 levels resulted similar to those of U937 cells but in anti-GRK3 clones they were increased by 50% and 70%. These results suggest that increased GKR2 levels may account for the H2R response in anti-GRK3 clones and further support the role of GRK2 in H2R desensitization. Present findings support that in U937 cells GRK6 is not involved in H2R desensitization and that GRK3 modulates GRK2 levels.

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Control of Basophil Functions through Histamine and Other Biogenic Amines

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Murine bone marrow comprises a population of basophil precursors, which upon stimulation display the typical pro-Th2 cytokine profile of this lineage by producing interleukin (IL)-4, IL-6 and IL-13 concomitantly, together with histamine. They can also take up histamine from the environment, a process that involves none of the classical histamine receptors but occurs through a bidirectional transport system that we have identified as Organic Cation Transporter 3 (OCT3). Indeed, *Oct3* mRNA is expressed in purified basophils derived from the bone marrow and typical substrates of the transporter inhibit their histamine uptake, which failed to occur in *Oct3*$^{-/-}$ mice. We show that, similarly to histamine itself, substrates of OCT3 diminish IL-3-induced histamine, IL-4 and IL-6 production by basophils from wild type but not from *Oct3*$^{-/-}$ mice. The inhibitory effect of the drugs tested depends on intracellular histamine since it was strikingly reduced in mice in which the gene encoding histidine decarboxylase had been disrupted, while exogenous histamine retained its activity, which proves that intracellular histamine levels must reach a critical threshold to induce the negative feedback signal. In accordance with the bidirectional functions of the transporter, intracellular histamine levels were strongly increased in IL-3-induced basophils from *Oct3*$^{-/-}$ mice, explaining why they generate less cytokines than their wild-type counterpart. We postulate that pharmacological modulation of OCT3 might become instrumental in the control of basophil functions during allergic diseases. We provide evidence that other biogenic amines, such as serotinin, can participate in the modulation of basophil activity.

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In Vitro and *in-Vivo* Stimulatory Potential of Bone Marrow-Derived Mast Cell on Tumor Growth

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Mast cells promote tumor development through many different ways: they could facilitate tumor angiogenesis through heparine-like molecules, and heparine could further permit neovascularization and metastases through its anti-clotting effects. Mast cells also generate and secrete IL-8, which is an angiogenesis factor as well as a tumor cell chemotactic factor and tumor mitogen. They also secrete histamine that could induce tumor cell proliferation through H1 receptors identified in malignant carcinoma, while suppressing the local immune response via H2 receptors.

Ongoing experiments in our laboratory use the HDC KO bone-marrow derived mast cells (histamine-deficient BMMCs) to investigate if the mast cells can induce mammary gland tumors.

We tested for the potential contribution of mast cells to breast cancer cell tumorgenesis in vivo syngeneic model. LM2 cells gave rise to tumors significantly more rapidly both in wild-type or HDC KO BALB/c mice when they were inoculated s.c. together with mast cells (wild-type BMMCs or HDC KO BMMCs too). All mice were sacrified 28 days after cell inoculation and the IL-10 and IL-12 expression was measured by QRT-PCR.

Only the wild-type mast cells induced the expression of IL-10 in the tumors, but the IL-12 was elevated in the presence of either HDC KO or histamine secreting BMMCs. Mast cells also promote cell growth in *in vitro* co-culture.

These result confirm that mast cells are effective in promoting the growth of mammary adenocarcinoma in vivo. Further evidences are also provided suggesting that histamine is one of the mediators playing critical role of the tumor-promoting effect of mast cells.

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Substantial Differences in the Kinetics of Histamine Release from Human Basophils Caused by Varying Strengths of IgE-Dependent Activators

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IgE-mediated histamine release from basophils and mast cells crucially contributes to the immediate symptoms of allergic disease. However, histamine secretion is suppressed at high allergen/anti-IgE concentrations, giving rise to a bell-shaped dose-response curve. Our aim was to characterise the mechanisms responsible for this apparent endogenous control of FcεRI-triggering. Human basophils were purified by Ficoll-density centrifugation, elutriation and negative selection using MACS. Histamine releases were assessed spectrofluorometrically and lysed cells were Western blotted to determine signal activities. Supraoptimal stimulation with either anti-IgE or the parasitic antigen IPSE led to lower mediator releases than optimum titrations but histamine release kinetics were much faster. In parallel, various signalling components in basophils (syk, p38 MAPK, ERK1&2) were more rapidly phosphorylated by greater concentrations of IgE-dependent stimuli, but abruptly deactivated after supraoptimal stimulation. This endogenous regulation involved the SH2-containing inositol 5' phosphatase (SHIP), which was highly phosphorylated following supraoptimal triggering compared to lower stimulus concentrations. Kinetics of IgE-mediated signalling and mediator release in FcεRI+ cells therefore varies substantially according to the magnitude of stimulation and SHIP plays an important role in the cessation of these events. The rapidity of allergic symptom generation may depend on the degree of IgE-receptor triggering, which is down-regulated by SHIP, a potential target for allergy therapy.

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The IL-4-Inducing Principle from *S. mansoni* Eggs (IPSE) Activates Human Basophils *via* a Novel Mechanism: "IgE Receptor Engagement without Crosslinking"

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The egg stage of the parasitic helminth *Schistosoma mansoni* leads to a Th2 immune response and IgE synthesis in the host. Investigating the underlying mechanism, we have recently identified a protein from extracts of *S. mansoni* eggs, called IPSE, which triggers human basophils from non-sensitised donors to release the key Th2/IgE inducer cytokines interleukin-4 (IL-4) and IL-13. Since IPSE turned out to be an immunglobulin-binding factor (Western blotting, Biacore analysis), our objective was to further characterise its mechanism of action. Functional tests with natural and recombinant IPSE – the latter in non-glycosylated as well as in glycosylated form - revealed that IPSE binds to both the Fab and the Fc part of the immunoglobulin molecule. Basophil IgE stripping and resensitisation experiments demonstrated that IPSE activates human basophils in an IgE-dependent fashion, however in contrast to other known IgE-binding factors, such as antigens, lectins or conventional B cell superantigens, it is not able to crosslink IgE. This was demonstrated by a variety of assays such as gel precipitation, sandwich blotting and Biacore analysis. Collectively, these data indicate that IPSE activates human basophils *via* a novel mechanism to release Th2 cytokines: "IgE receptor engagement without crosslinking". Since basophil IL-inducing activities are present also in extracts from other parasitic helminths like *E. multilocularis*, we suppose they might represent a general immunomodulatory principle common to parasitic worms.

Accumulation and Activation of Basophils in Allergic Disease

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Basophils can play key roles in allergic conditions by releasing potent mediators of inflammation, including histamine, proteases, eicosanoids, and a range of cytokines (including IL-1β, IL-4, IL-5, IL-6, TNF-α and IFN-γ). There is considerable heterogeneity in the products stored and secreted from basophils, with variation between subjects (e.g. in the forms and quantities of tryptase); and in the same subjects there can be marked differences in cytokine expression between basophils in the circulation and those in different tissue compartments. Immunohistochemistry with the basophil-specific antibody BB1 has revealed increased numbers of these cells in the lower airways of subjects with asthma (especially in fatal cases), in the upper airways in rhinitis, and in affected tissues in other allergic conditions. The antigen recognised by BB1 has been termed *basogranulin* on account of its selective presence in the secretory granules of basophils. It is a highly basic protein (pI of 9.6) with a molecular weight of 124 kDa on Western blotting, and is secreted from basophils in a massive complex of some 5,000 kDa. Basogranulin is released from basophils in parallel with histamine and tryptase in response to various stimuli *in vitro*. Its detection in nasal lavage and other fluids from subjects with allergic conditions is now providing direct evidence for the activation of basophils in disease. Basogranulin should be useful as a clinical marker for basophil activation and could represent a new mediator of allergic disease.

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Old and Novel Mediators of Th2-Type Immune Responses and Allergy Produced upon Mast Cell – Basophil Interactions

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Current views of the pathogenesis of immediate-type hypersensitivity diseases emphasize the role of mast cells in the immediate response to allergen, while APC's, Th2-lymphocytes and eosinophils are regarded as the major players in late-phase reactions and chronic allergic inflammation. Here we propose that human allergic inflammation is predominantly a mast cell – basophil affair which is triggered by allergen specific IgE followed by attraction and activation of basophils: Indeed, together they produce high levels of all the mediators and cytokines which have been implicated in allergic inflammation (e.g. Histamine, PGD2, LTC4, GM-CSF, IL-3, IL-4, IL-5, IL-13, NGF, various chemokines). The major regulators of this co-operation are basophil-derived IL-4 and mast cell-derived IL-3. Using genomic and proteomic approaches we also identified two novel potential mediators of allergy which are formed when blood basophils are exposed to human mast cells which have been activated by IgE-receptor crosslinking. Supernatants of IgE-activated mast cells (or IL-3) were found to qualitatively change the make-up of granules of human basophils by inducing *de-novo* synthesis of granzyme-B, but not other lympoid granule proteins. This IL-3 effect is restricted to basophil granulocytes and not shared by other cytokines known to act on basophils or lymphocytes. Induced granzyme-B is sorted into the granule compartment and released during exocytosis triggered by IgE-dependent and –independent agonists. *In vitro*, there is a close parallelism between granzyme-B, IL-13 and leukotriene generation. *In vivo*, granzyme-B, but not the lymphoid granule marker granzyme-A, is released after allergen challenge of asthmatic patients in strong correlation with IL-13, suggesting a role of granzyme-B as a novel mediator of allergic diseases. Furthermore, exposure of basophils to activated mast cells leads to the induction of an enzyme generating a novel lipid mediator which not only modulates the phenotype of basophils in an autocrine manner but also affects the expression of homing receptors and cytokine profiles of T-helper cells. Thus, this co-operation between mast cells and blood basophils could orchestrate the cellular and functional alterations found in allergic diseases and other Th2-type immune responses such as infections with helminthes.

"British Diet on a Chip": A Novel Diagnostic Tool for the Detection of Food Allergy Combining Protein Microarrays with human Basophils

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The cornerstone of modern allergy diagnosis is still the skin prick test, which has been used for over 100 years. This test is less amenable to food allergens, as many food allergens do not have the required stability. UniCAP RAST tests detect the presence of allergen specific IgE in serum. CAST tests detect basophil-derived leukotrienes, but are expensive and difficult to interpret.

Our objective is to develop a Protein Microarray System (PMS) containing most of the proteins from the British diet as a powerful alternative to costly or labour-intensive diagnostic tests such as UniCAP or ELISA-based assays for the detection of allergen-specific IgE. In a further step, we have modified and optimized the PMS by including live human basophilic granulocytes. Our intention is to couple the diversity and power of the protein array, with the sensitivity of the basophils, adding a new biological dimension to the test.

Basophils are purified to homogeneity from peripheral blood of healthy donors, briefly treated with lactic acid to remove receptor-bound IgE (IgE stripping), and resensitized with the serum or IgE preparation to be tested. The basophils newly loaded with IgE are then coincubated with the protein arrays. Basophil activation, indicating effective crosslinking of IgE by allergens, is monitored via upregulation of specific basophil activation surface markers (i.e. CD63 and CD203c). We have compared different types of protein microarray slides (Fast slides, aldehyde slides), as well as the basophilic cell line KU-812 and peripheral blood basophils. In our preliminary experiments, a set of allergens, control proteins or anti-IgE antibody were spotted on protein array slides by hand. Purified basophils or KU-812 cells were incubated in different densities for various periods of time, the slides washed with isotonic buffer, and cell binding visualized by light microscopy.

Among the different microarray slide materials tested, the Fast slide showed the highest basophil binding. KU-812, even when pretreated with 2ng/ml IL-4 for 21 days, only exhibited weak binding, probably due to the low surface expression of the high affinity IgE receptor FcεRI. KU-812 displayed unspecific binding to mammalian albumins. Purified peripheral basophils displayed strong binding to anti-IgE antibody and relatively low unspecific binding. Basophils stripped and resensitized with the serum of a grass pollen allergic patient exhibited specific binding to the grass pollen extract.

Our data show that a technology which combines allergens on a protein microarray chip with living purified basophils is feasible in principle. In the long term, peripheral blood basophils will have to be replaced with a suitable cell line, making the costly, cumbersome and time-consuming basophil purification redundant. This system will not only facilitate the identification of potential food allergies, but can be extended to other (e.g. respiratory) allergens or to the differential diagnosis of human helminth infection.

POSTER PRESENTATIONS

P 1

H_3 Receptor Activation Phosphorylates Akt in Rat Cortical Neurons

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H_3 receptor (H_3R) activation in transfected cells modulates several intracellular events such as inhibition of adenylate cyclase (Lovenberg et al. Mol Pharmacol 1999; 55:1101-1107), MAPK phosphorylation (Drutel et al. Mol Pharmacol 2001;59:1-8) and Akt phosphorylation (Bongers et al, 2004 33rd EHRS Meeting, Koln). Here we demonstrate that in rat embryonic day (ED)17 cortical neurons activation of the H_3R induces a time dependent phosphorylation of Ser^{473} Akt. Significant effects were determined by ANOVA and Bonferroni's test.

We first showed that ED 17 cortical neurons maintained in cultured for 4, 7 or 11 days stably expressed H_3R mRNA. Incubation of cortical neurons with histamine (HA, 100 nM), the H_3R agonists R-α-methylhistamine (RAMH, 100 nM) or immepip (10 nM) induced phosphorylation of Ser^{473} on Akt reaching a maximum at 60 min of incubation. Akt activation by RAMH was inhibited by the H_3R antagonist thioperamide (300 nM) and by LY294002 an inhibitor of PI3 kinase. Furthermore, RAMH failed to activate CREB, which is one of the downstream effectors of Akt.

Phosphorylation analysis of signaling proteins is fundamental to examine intracellular signaling pathways. Akt activation, besides promoting neuronal survival, regulates synaptic strength and is critical for memory consolidation and retrieval. On the other hand, there is ample evidence that H_3R ligands may have either beneficial or detrimental effects on learning and memory. Identifying the intracellular signaling properties of H_3R in the brain may help understand the mechanisms by which histamine and H_3R ligands affect cognitive functions.

Histamine H_3 Receptors Modulate [^{3}H]-Dopamine Release in Rat *Substantia Nigra Pars Reticulata*, but not in Prefrontal Cortex.

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Through the nigro-striatal and meso-cortical systems, dopamine plays an important role in regulating the function of basal ganglia and prefrontal cortex (PFC). Whereas basal ganglia participate in the control of motor activity, PFC is involved in emotional behaviours [1].

Histamine H_3 receptors are present in both PFC and *substantia nigra pars reticulata* (SNr) [2], the latter being part of the basal ganglia, and the present study was designed to elucidate the role of H_3 receptors in the control of dopamine release in these regions.

Binding studies revealed that the H_3 selective radioligand N-α [methyl-^{3}H]-histamine ([^{3}H]-NMHA) labelled a single saturable population of sites in membranes from SNr slices (Bmax 648 $\pm$ 50 fmol/mg protein, Kd 0.58 $\pm$ 0.06 nM) and PFC synaptosomes (Bmax 349 $\pm$ 26 fmol/mg protein, Kd 0.14 $\pm$ 0.01 nM). In both regions [^{3}H]-NMHA binding was inhibited with high potency by the H_3 agonist immepip and the H_3 antagonist clobenpropit.

In superfused rat SNr slices depolarisation-evoked [^{3}H]-dopamine release was diminished by immepip (100 nM, 50 $\pm$ 5% reduction). In contrast, immepip failed to affect [^{3}H]-dopamine release from PFC synaptosomes.

Our data indicate a differential control by H_3 receptors of dopamine release in two brain regions which differ in the origin of their dopaminergic innervation.

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P 3

Is the High Affinity Selective H_3 Receptor Agonist Methimepip Anxiolytic or Anxiogenic in a New Validated Animal Models of Human Anxiety?

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Growing evidence has suggested a role for the histamine H_3 receptor (H_3R) in cognitive and emotional responses, however, there is conflicting reports of whether the H_3R plays a role in anxiety. We suggest that one probable reason for these inconclusive reports, is the paucity of validated animal models for human anxiety. Exposure to novelty has been shown to induce anxiety responses in a variety of behavioral paradigms. However, most current designs of animal models of human anxiety offer mixed settings in which avoidance responses are encouraged over risk-taking responses. How much anxiety is there if a safe alternative is available? We have developed the first simple validated animal models for human anxiety (Ennaceur *et al*., 2006a, b), where there is no safe alternative.

In the present study, we examined the behavior of mice in a novel 3D maze, composed of eight flexible arms radiating from a central platform. Each arm can be manipulated independently and presented at the same, below or above the level of a central platform. Mice need to cross a bridge to reach flat, raised or elevated arms. Naïve mice were tested for the first time in the raised arm configurations of the maze. The effects of different doses of the H3R selective agonist methimepip (Kitbunnadaj *et al*., 2005) are explored on the behavior of Balb/c mice which have been previously shown to be more anxious than C57 mice and less anxious than C3H mice (Ennaceur *et al*., 2006b). The results of this model showed that exposure for the first time to the raised arm configuration increases the latency of entry to arms and decreases the number of visits to the bridges and the arm of the maze in Balb/c and C3H mice in comparison to C57 mice. Therefore, the level of anxiety expressed by the three mice strains range from high (C3H), intermediate (Balb-C) to low (C57).

An anxiolytic effect of methimepip will align the behavior of Balb/c to C57 mice, and an anxiogenic effect of methimepip will align the behavior of Balb/c to C3H mice. This model will be an ideal tool to determine, for the first time, the role of the H_3 receptor in anxiety.

Ennaceur A, Michalikova S, Wilson ST, Chazot PL. Behav Brain Res 2006a (submitted)
Ennaceur A, Michalikova S, Wilson ST, Chazot PL. Behav Brain Res 2006b (submitted)
Kitbunnadaj R, Hashimoto T, Poli E, Zuiderveld OP, Menozzi A, Hidaka R, de Esch IJ, Bakker RA, Menge WM, Yamatodani A, Coruzzi G, Timmerman H, Leurs R. J Med Chem 2005;48:2100-7.

The presenting author wishes to thank the Wellcome Trust (UK) for financial support. Dr Ennaceur is a recipient of an RDF fellowship from the University of Sunderland for 2005-2006.

Down-regulation of Histamine H_3 Receptors in Rat Striatum

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In this work we took advantage of the postsynaptic location of histamine H_3 receptors on the somata of striatal GABAergic neurones [1,2] to study whether agonist activation leads to the desensitisation of native H_3 receptors.

Pre-incubation of striatal slices with the selective H_3 agonist immepip (100 nM, 5-30 min) decreased by ~50% the specific binding of *N*-α-[methyl-^{3}H]-histamine ([^{3}H]-NMHA) to membranes obtained from the treated slices, with partial recovery (~40%) after 90-min washout. The immepip effect was mimicked by histamine and the H_3 agonists imetit and *R*-α-methylhistamine, and was blocked by the H_3 antagonist thioperamide. Saturation analysis showed a decrease in Bmax (-40 ± 9%) and a two-fold increase in the dissociation constant (K_d 1.15 ± 0.23 versus 0.59 ± 0.17 nM).

The immepip-induced decrease in [^{3}H]-NMHA binding was fully and partially prevented by incubation at 4°C and in hypertonic medium respectively, but not by the endocytosis inhibitor phenylarsine oxide (10 μM). Further, the effect of immepip was not blocked by any of the following protein kinase inhibitors: Ro-318220 and Gö-6976 (PKC, 200 nM), H-89 (PKA, 1 μM) and staurosporine (general inhibitor, 300 nM).

Pre-incubation with immepip (100 nM, 15 min) of [^{3}H]-adenine-labelled striatal slices prevented the inhibitory effect of H_3 receptor activation on forskolin-induced [^{3}H]-cAMP accumulation (99 ± 9% versus 76 ± 4% of controls).

Our results indicate that agonist binding promotes the down-regulation of striatal H_3 receptors resulting in significant loss of function.

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P 5

Pharmacological Characterization and *in vitro* ADME Properties of Immethridine, a Potent and Highly Selective Histamine H_3-R Agonist

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Since the discovery of the Histamine H_3-receptor (H_3R) [1], and the cloning of human H_3R [2], numerous distinctive H_3R properties have been revealed (constitutive activity, interspecies differences, multiple splices isoforms, protean agonism) and influenced the whole H_3R drug discovery process in the last 5 years. The discovery of novel imidazole-based H_3R agonists and antagonists, followed by non-imidazole H_3R antagonists and inverse agonists, by academic research groups and many pharmaceutical companies, reflected the gain of interest to better understand the H_3R and its implication in treatment of PNS- and CNS–related disorders [3]. Highly selective H_3R ligands are therefore required to evaluate and validate novel therapeutic approaches. The present study described the pharmacological characterization and in vitro ADME properties of H_3R agonist Immethridine. Immethridine represent a novel, highly potent, histamine receptor-subtype selective, H_3 agonist (pKi hH_3R= 9.1 and pEC_{50} = 9.1) with around 500-fold selectivity over the recently identified human histamine H_4 receptor (H_4R) (pKi hH_4R = 6.4). Immethridine (at 10µM) displayed no significant interactions with a large panel of GPCRs, ions channels and enzymes. Its selectivity ratio for H_3R was still around 1000-fold compared to its affinity for β_2-adrenergic receptor. Immethridine and immepip were equipotent and equiactive on the three rat H_{3A} (445), H_{3B} (413) and H_{3C} (397) receptor isoforms tested in the functional assays. Furthermore, despite its imidazole moiety, Immethridine showed moderate inhibition of human CYP2C9, 2C19, 2D6 and 3A4 isoforms as well as a good *in vitro* metabolic profile on both rat liver microsomes and hepatocytes. Finally, Immethridine significantly decreased histamine levels in the rat prefrontal cortex at a dose of 10 mg/kg (ip administration). Immethridine is a novel, highly potent and selective histamine H_3R agonist with good *in vitro* ADME and physicochemical properties. This new pharmacological tool is now available to the scientific community to study the role of the histamine H_3 receptor at a molecular level.

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[2] Lovenberg TW et al. Mol Pharmacol 1999;55:1101
[3] Celanire S, Wijtmans M, Talaga P, Leurs R, de Esch I. Drug Discovery Today 2005;10:1613

Histamine H_3 Receptors Regulate Glutamate, but not GABA Release in Rat Thalamus

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The thalamus is involved in the processing by the brain of sensory and motor information. Thalamic function is mostly based on excitatory glutamatergic and inhibitory GABAergic transmission [1], and the aim of this work was to determine the presence of histamine H_3 receptors on thalamus nerve terminals and the effect of their activation on glutamate and GABA release.

N-α-[methyl-^{3}H]histamine ([^{3}H]NMHA) binding to membranes from rat thalamus synaptosomes (Bmax 141 $\pm$ 12 fmol/mg protein, K_d 0.78 $\pm$ 0.20 nM) was inhibited by histamine and the H_3 agonist immepip (two-site model) as well as by the H_3 antagonist clobenpropit (one-site model). GTP-γ-S (30 μM) decreased [^{3}H]NMHA binding by 55 $\pm$ 4% and made the histamine inhibition fit better to the one-site model. Immepip (30 nM) induced a modest, but significant increase (113 $\pm$ 2% of basal) in [^{35}S]-GTPγS binding, an effect prevented by clobenpropit (1 μM) and by pre-treatment with pertussis toxin.

Depolarisation-induced glutamate release from thalamus synaptosomes was inhibited by immepip (30 nM, 38 $\pm$ 7% reduction), an effect prevented by clobenpropit (1 μM). Conversely, immepip (up to 1 μM) had no effect on depolarisation-evoked [^{3}H]GABA release. In corticothalamic slices H_3 receptor activation produced inhibition (38 ± 7%) of the glutamate receptor-mediated field potentials (FPs), and an increase in the FP2/FP1 ratio (from 0.86 ± 0.03 to 1.38 ± 0.05) in a paired-pulse paradigm.

Our results demonstrate the presence on thalamic nerve terminals of H_3 receptors coupled to Gαi/o proteins, whose activation modulates glutamatergic, but no GABAergic transmission.

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P 7

Heterocyclic Replacements for the Central Phenyl-Core of Diamine–Based Human Histamine H_3 Receptor Antagonists

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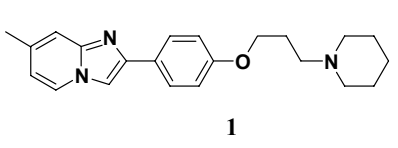
In earlier publications we have presented the SAR for diamine-based histamine H_3 antagonists (1-4), [1-5], together with a proposed pharmacophore [3, 5] consisting of a central phenyl core flanked by two basic moieties. This model has in turn evolved to account for the numerous non-imidazole based histamine H_3 antagonists discovered in both our own laboratories and the laboratories of other groups [6]. The primary objective of our initial research was to delineate the SAR of the two basic components whilst maintaining a central phenyl core. With the information gained from these studies we turned our attention to replacement of the phenyl component with a range of 5- and 6-membered ring hetero-aromatic fragments. This led to the identification of several series of potent H_3 antagonists [7, 8] whose synthesis, SAR and biological data will be presented.



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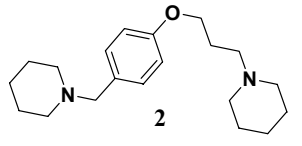

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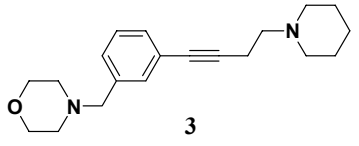

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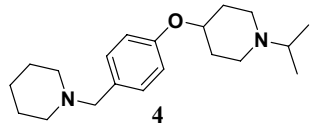

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Histamine 3 Receptor Inverse Agonists for the Treatment of Obesity. Biological and Chemical Challenges

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There is a rising prevalence of obesity throughout the world. Obesity is a major risk factor in the development of conditions such as hyperglycemia, dyslipidemia, insulin resistance, coronary artery disease and cancer.

The Histamine receptor family currently consists of 4 subtypes. The Histamine H_1 and H_2 receptors have been already extensively studied, leading to the identification of very valuable medicines. While the discovery of H_4 receptor is relatively recent and its usefulness is still under investigations, the H_3 receptor is under the spot lights since several years. The Histamine H_3 receptor, as a molecular target predominantly expressed in the Central Nervous System (CNS), might present opportunities in two main fields: the Cognitive diseases (*e.g.* Alzheimer, Depression, Anxiety and Schizophrenia) and Metabolic diseases (*e.g*. Obesity, Dyslipidemia).

Being inspired by High Throughput Screening, Public Information Driven Approach and Pharmacological Models, the authors would like to present the generation of several novel hits structures *in F. Hoffmann-La Roche*. A specific screening cascade has allowed the rapid optimization of these hit structures to high affinity, metabolically stable, and brain penetrating compounds. A dipsogenia experiment and a modified Irwin screen are included early in the profiling in order to show *in vivo* receptor efficacy and to detect potential CNS-mediated or unspecific effects, respectively. Using some of these hit compounds, validation of the Histamine H_3 receptor was performed in several obesity models and details will be presented, together with Structure Activity Relationships and refinements towards the identification of a promising lead compound

P 9

Ureas with H_3-antagonist Activity – A new Scaffold Discovered by Lead-hopping Starting from Cinnamic Acid Amides

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An earlier detected structure class of H3-antagonists, the cinnamic acid amides, exemplified by NNC 0038-0000-1202 [1], bears the potential risk of chemical and metabolic instability due to the polar double bond in cinnamic acid. Moreover, many of these compounds had shown substantial hERG-channel inhibition. Therefore, lead-hopping was used to propose the replacement of the C=C-double bond in the cinnamic amide with a NH-C- single bond, leading to a new compound class with urea structure.

Parallel synthesis on solid phase or in solution was employed to generate libraries of tri- or tetrasubstituted ureas bearing similar substitution as the original cinnamic amides. More than 500 compounds were synthesized in several design cycles, leading to potent antagonists at the human H3-receptor. The routes of synthesis and some *in vitro*- characteristics of selected compounds will be presented. One of the best examples was [4-(3-aza-bicyclo[3.2.2]nonane-3-carbonyl)-piperidin-1-yl]-(4-isopropyl-piperazin-1-yl)-methanone (Ki = 7 nM at the cloned human H3-receptor; hERG-planar patch clamp IC50 >10 microM).

It could thus be shown that lead hopping from cinnamic amides to analogous ureas was successful and a number of potent H3-antagonists with negligible hERG inhibition could be identified.

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P 10

Radiosynthesis and Biodistribution of a Histamine H_3 Receptor Antagonist: Evaluation of a Potential PET Ligand

Anu J Airaksinen[1], Jill A Jablonowski[2], Margreet van der May[1], Ann J Barbier[2], Rob P Klok[1], Joost Verbeek[1], Robert Schuit[1], Jacobus DM Herscheid[1], Josee E Leysen[1], Nicholas I Carruthers[2], Adriaan A Lammertsma[1], Albert D Windhorst[1]

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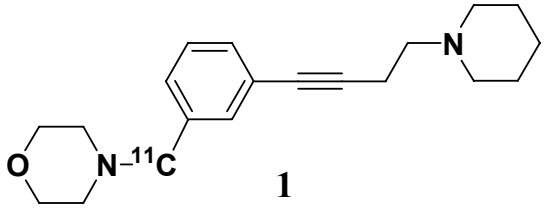
The potent histamine H_3 receptor antagonist, piperidine alkyne **1**, was successfully labeled with ^{11}C, in a novel one pot reaction sequence, in high chemical yield (decay corrected yield 28 ± 8 %), and with high specific radioactivity (56 ± 26 GBq/μmol). Binding of [^{11}C]**1** to H_3 receptors was studied *in vitro* in rat brain and *in vivo* in rats and in mice. [^{11}C]**1** was shown to be a highly selective ligand for H_3 receptors *in vitro*. *In vivo* [^{11}C]**1** showed high brain uptake and good selectivity for H_3 receptor-rich brain regions in rats and mice. The evaluation of [^{11}C]**1** as a potential histamine H_3 receptor PET ligand will be presented.



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P 11

(3-Piperidin-1-yl-Propoxy)-Tetrahydroisoquinolines and Tetra-hydro-azepines: a Novel Series of Selective Histamine H_3 Receptor Antagonists

Cynthia D Jesudason, Lisa S Beavers, Jeffrey W Cramer, Joelle Dill, Don R Finley, Scott D Gleason, Susan K Hemrick-Luecke, Craig W Lindsley[1], David LG Nelson, F Craig Stevens, Robert A Gadski, Samuel W Oldham, R Todd Pickard, Christopher S Siedem[1], Dana K Sindelar, Ajay Singh[1], Brian M Watson, Jeffrey M Witkin, Philip A Hipskind

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A novel series of selective, potent histamine H3 receptor antagonists (H3R) was synthesized around the tetrahydroisoquinoline and tetrahydroazepine platforms. The goal of the project was to develop a potent antagonist that was selective only for H3R and not the other known histamine receptor subtypes H1R, H2R, and H4R. The substitution around these rings as well as the nature of the substituent on nitrogen was explored. As reported in the literature we also saw a species difference with all the compounds being less potent at the rat receptor. We selected two compounds based on their high affinity for human H3 receptors for further in vivo evaluation. These compounds potently inhibited the ex vivo binding of [3H]-R-alpha-methylhistamine in rat brain homogenates (IC50 = 1.1 and 5.3 mg/kg, po,). The oral bioavailability of these compounds in rat after a dose of 10mg/kg was found to be >50%.

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P 12

Mast Cell-Derived Interleukin-8 May be Involved in the Ovarian Mechanisms of Follicle Growth and Ovulation

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Changes of mast cells (MC) content and distribution in ovarian tissue during the estrus suggest an involvement of MC in local regulation of ovarian function. Based on in vivo studies, interleukin-8 (IL-8) can also be considered an important regulatory factor in the ovary. Ascitic fluid of patients with ovarian hyperstimulation syndrome contained high IL-8 concentrations [1]. Number of IL-8 positive cells is subjected to changes during the estrus and corresponds with MC [2].

We examined influence of IL-8 on the development and ovulation of ovarian follicles cultured in vitro.

Preantral follicles (N=120) of 150-180μm obtained from immature mice were individually cultured in medium containing 5% immature mouse serum, supplemented with 100 mIU/ml recombinant human FSH, as method described in ref [3]. Diameter of the follicles was measured daily using x100 magnification and a calibrated micrometer. Starting from Day 3, the medium was supplemented with IL-8 (100 ng/ml) or IL-8 and blocker of IL-8 receptors CXCR1/R2 (repertaxin; 100nM) in Group I and II, respectively. The follicles in Group III were cultured without IL-8 and/or repertaxin. At the end of culture period (5-6 days), follicles from Group II and Group III were induced to ovulate with hCG (5 IU/ml). In Group I ovulation rate (OR) was estimated in subgroups: IA - without stimulation by hCG, IB – with hCG. The mean daily increase in follicular size in Group I amounted 29.87 ±5.34 (% ±SD) and was significantly higher, compared to Group II and III (18.75 ±4.33 and 19.16 ±4.12, respectively). The highest OR observed in the IB was higher than the OR values in Group II and III (27% vs. 15% and 17%, respectively; $p<0.05$). OR in the IA was significantly lowered and amounted 8%. We conclude, that IL-8 can stimulate follicular growth and acting together with hCG, increases OR.

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P 1 3

Potent Histamine-Releasing Activity of Atrahagin, a Snake Venom Metalloproteinase

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Earlier studies found that cobra venom induced mast cell degranulation and histamine release from rat diaphragm and peritoneal mast cell suspensions, and isolated guinea pig lung mast cells.

Atrahagin was purified using heparin–sepharose followed by Superdex 75 gel filtration chromatography from the venom of Chinese cobra (*Naja atra*). Human mast cells were enzymatically dispersed from colon, lung and tonsil. The dispersed cells were then cultured with atrahagin, anti-IgE or calcium ionophore A23187 (CI), and the histamine release was determined.

A novel metalloproteinase atrahagin in Chinese cobra (*Naja atra*) snake venom was purified, using heparin–sepharose followed by Superdex 75 gel filtration chromatography. Apart from its α-fibrinogenase activity, atrahagin potently activated human colon, lung and tonsil mast cells with the net histamine release being 25.9$\pm$4.4, 17$\pm$1.9, 13.2$\pm$3.6%, respectively. Time course study revealed that the peak histamine release induced by atrahagin occurred at 12, 12, and 8 min following incubation of the enzyme with colon, lung and tonsil mast cells, respectively. The response of mast cells to atrahagin was abolished by preincubation of cells with metabolic inhibitors or pertussis toxin, and by removal of Ca^{2+} and Mg^{2+} from the challenge buffer.

Activation of human mast cells by atrahagin indicated that the enzyme might contribute to the pathogenesis of snake wound.

Gene Expression Profiling of Mouse Mucosal Mast Cell (mMMC) Proteases

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Mast cells store histamine, cytokines, proteoglycans and proteases in their granules which are released in the extracellular space upon stimulation. In the mouse, connective and mucosal mast cells can be distinguished based on their tissue distribution and immunohistochemical characteristics.

In our studies we followed the gene expression changes during mMMC differentiation (in the presence of TGF-βI) by oligo microarrays. Microarray data were clustered and then protease and protease inhibitor genes were further analysed. While the expression of Mcpt-5, -6, transmembrane tryptase (TT) and Spink2 was elevated in the early phase, HtrA1 and Mcpt-1 mRNA level increased only in the final stage of maturation. Real-time PCR data confirmed our microarray results. After IgE/antigen-induced stimulation, the expression of TT and Mcpt-1 was enhanced, Spink2 level remained unchanged and there was a marked decrease in HtrA1 production. By comparing different mast cell populations, HtrA1 seemed to be a mucosal mast cell-specific protease. The characteristic changes in HtrA1 expression were confirmed by immunoblot, too. Furthermore, cord blood-derived human mast cells were positive for HtrA1.

Our data show that mucosal mast cells tightly regulate their protease expression during the differentiation and a characteristic protease/inhibitor set is found in the immature precursor form. HtrA1 contains not only a serine-protease, but a Kazal-type inhibitor, an IGFBP-like and a PDZ domain, too. It can cleave extracellular matrix components (fibronectin, collagen) and functions as a TGF-β-inhibitor. In different metastatic tumors (melanoma, ovarian cancer) its expression is strongly down-regulated. Based on these data it can be assumed that HtrA1 plays an important role not only in the extracellular matrix remodelling initialized by mucosal mast cells, but it may also inhibit the TGF-β-induced final mast cell maturation, thus providing a nagative feedback.

P 15

Distribution of Mast Cell Tryptase and Metallothionein in Human Brains with Amyloid Deposit

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The classical pathological lesions that characterize Alzheimer`s disease (AD) are brain amyloid deposit (senile plaques) and neurofibrillary tangles. However, a substantial loss of neurons and synapses in affected brain areas better correlates of dementia than the classical AD markers. A variety of different mechanisms has been suggested to contribute to neuronal death in AD, including genetic and environmental factors. Increasing data support the hypothesis that inflammation is involved in the pathogenesis of disease. Mast-cell tryptase is a protease with proinflammatory activity and an important indicator of mast cell activation and degranulation. Metallothionein (MT) has several putative roles in metal detoxification, in scavenging free radicals, and in acute phase response. Therefore we have studied the distribution of these two proteins in the brains of individuals with AD and in brains of patients who died several days or weeks after resuscitation.

Brain samples were fixed in 4% paraformaldehyde and serial sections were used for immunohistochemistry. Primary antibodies generated against different domains of amyloid beta protein, metallothionein, mast cell-tryptase, and antibodies that recognised different types of glial cells were included in the study.

In all AD brains amyloid plaques were numerous but they were also present in brains of some nondemented, aging patients after resuscitation. Numerous, shrunken and very small tryptase positive cells were scattered through-out the tissue but always they were located in the vicinity of the brain blood vessels and capillaries. Metallothionein was detected in astrocytes and in cells of brain vessel walls. In cerebral cortex with amyloid deposit metallothionein was present in astrocytes and outside of cells.

To our knowledge, this is the first report indicating that brains with amyloid deposit are infiltrating by tryptase positive cells and that astrocytes in these brains may play a role in scavenging free radicals.

P 16

Clinico-biological Characteristics of Flow Cytometry Applied to NSAIDs Hypersensitivity Diagnosis. Determination of Preliminary ROC Curves

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Non steroidal anti-inflammatory drugs (NSAIDs) induced hypersensitivities are frequently explained by the inhibition of cyclo-oxygenase. In order to study these reactions, we applied flow cytometry (IgE/CD63 method), to the analysis of NSAIDs induced basophil activation and demonstrated that NSAIDs were capable of specifically induce a CD63 expression in patients having presented NSAIDs induced hypersensitivities.

In order to obtain the main characteristics of this method we selected 13 patients having experienced an immediate (<1h and in 12 cases 15 min) cutaneous reaction (urticaria and/or angio-oedema (group1), 6 asthmatic patients having experienced an NSAIDs induced asthma AIA (group2) and 15 tolerant controls, having taken recently (<1 year) an NSAID without clinical reaction (group 3). Leukocytes were obtained by buffy coat , incubated with three dilutions of ibuprofen, lysin acetyl salicylate and ketoprofen in the presence of 2 ng/ml IL3 and results were expressed in index involving both CD63 expression and IgE/RIgE clustering. CD63 expression was observed in 5 patients of the group1 (38%) or 8/36 tests (22%) and not for groups 2 and 3. The mean index calculated for the 3 allergen concentrations tested were respectively for group 1 : 89+/-34, 39+/-77, 14+/-29, group 2 : 3,0+/-6.8, 3.6+/-4.7, 4.3+/-5.8 and group 3 : 2.1+/-4, 1.8+/-2.6, 1.2+/-1. Results observed for group 1 and 2, 3 were statistically significant (Wilkoxon rank test). The ROC curve calculated for a series of index ranging from 4 to 12 showed that the optimal positivity threshold was 12 with, respectively for ASA, Ibuprofen and ketoprofen sensitivities (0.38, 0.73, 0.60), specifities (1, 1, 0.93). The simultaneous use of ASA and ibuprofen would lead to a sensitivity of 0.75, specificity of 1.

These characteristics are in favour of the use of this flow cytometric method for the diagnosis of cutaneous reactions induced by NSAIDs.

P 17

Amitriptyline Inhibits Mast Cell Histamine Secretion: Implications for Chronic Fatigue Syndrome Therapy

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Chronic fatigue syndrome (CFS) is a disorder characterized by cognitive problems, dizziness, fever malaise, muscle aches, nausea and sleep disturbances. Neuroimmunondocrine interactions are suspected, but there are no effective treatments except for tricyclic antidepressants, the beneficial action of which is not understood. We hypothesized that this may be through inhibition of mast cell histamine secretion.

To investigate if the tricyclic amitriptyline could inhibit histamine secretion from mast cells.

Rat peritoneal mast cells (90% pure) were incubated with amitriptyline for 5 min, followed by 5 more min with the mast cell secretagogue compound 48/80 (1 µg/ml). Histamine and intracellular calcium ions were measured.

Histamine release was increased from 3.6% to 71±3% with 48/80 (1 µg/ml), but was inhibited significantly by amitriptyline in a dose-dependent manner to 11±2% at 1 mM. Stimulation by 48/80 increased intracellular calcium ion levels. Pretreatment with amitriptyline (0.1 mM) for 5 min significantly reduced the basal levels and this decrease could be overcome only by addition of much higher concentrations of triggers. Cell viability was intact.

These results indicate that amitriptyline can decrease mast cell intracellular calcium levels, thus preventing stimulated histamine secretion. The effect on other inflammatory mediators should also be examined, especially on brain mast cells.

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Lack of Endogenous Histamine Affects Early Inflammatory Phase of Wound Healing

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Investigation of the role of histamine in proliferation/regeneration has a long history, but currently it has become a hot topic again.

We have investigated cutaneous wound healing in histamine deficient HDC KO and WT mice. We intended to see, if lack of endogenous histamine would affect the early inflammatory phase of wound healing.

In animals fed by histamine-free food, excisional wounds of KO mice healed slower between the 1st-3rd days, the closure of the wound was 25% reduced compared to that of the WT animals. This difference has disappeared by the 7th day, regeneration has been completed by the 14th day. In animals, fed by regular food, we didn't find any difference between the experimental groups. Using QRT-PCR we investigated if the absence of endogenous histamine could influence the expression of the following genes (all of which are involved in inflammation): H1 receptor (H1R), H2 receptor (H2R), IL-6 receptor (IL-6R), gp130, TGFbeta1 (TGFb), TGFbeta receptor II (TGFb-R), integrin beta2 (*intb*) and villin2 (*vill*), the latter two acting on the migration of inflammatory cells. On the 3rd day of wound healing we found that the basically enhanced gene expression of IL-6R and TGFb was more elevated in wounds of KO mice, than in WT mice. Interestingly H1R and H2R gene expressions were upregulated in WT mice, but didn't change in KO mice. According to our results, early inflammatory phase of wound healing lasts longer in the absence of endogenous histamine, in parallel with elevated gene expression of receptor-complex subunits of inflammatory citokine IL-6, such as that of TGFb. In lack of histamine it seems there is a much more pronounced need for inflammation induction by IL-6, as well as for TGFb that is an inductor of collagen synthesis. We suppose that HDC KO mice would partly compensate the failure of early wound healing by the upregulation of collagen synthesis, thereby overtakes the delay for the 7th day.

P 19

Transtympanic *versus* Intramuscular Steroid Administration in a Histamine- Induced Inflammatory Middle Ear Model

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Assessment of the histopathologic effect of transtympanic and intramuscular administration of dexamethasone in a vivo experimental animal model of middle ear mucosal inflammation.

Fifty healthy rabbits weighting 1500-1800 gr were randomly divided in three groups. In 10 animals (control group) 0.5 ml histamine 20mg/ml was injected by using a 25-gauge needle transtympanically in the inferior-anterior quadrant of the right tympanic membrane. In 20 rabbits (group A) histamine challenge followed a three day intramuscular pretreatment with dexamethasone at 1mg/kg per day. In 20 rabbits (group B) histamine challenge followed pretreatment with dexamethasone through transtympanic route (0.3 ml – 1.2 mg dexamethasone). Middle ear mucosa was obtained for histopathology 30 minutes after histamine administration. Using a semi-quantitative 0-3 scale the following parameters were assessed: inflammation, acute inflammatory component, presence of eosinophils, activity of the inflammation and fibrosis.

Edema, vascular dilatation and congestion, inflammation, the presence of an acute (polymorphonuclear) inflammatory component, the presence of eosinophils and activity of the inflammation were found to be of a lesser grade in mucosae of the group B. All differences were found to be statistically very significant ($p<0.01$) using the Mann-Whitney test.

Our findings validate the transtympanic route of dexamethasone administration in counteracting histamine effects.

P 20

A Heretofore Undisclosed Crux of Eosinophilia-Myalgia Syndrome: Compromised Histamine Degradation

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In the 1980s two bewildering, life-threatening and noncontagious outbreaks of blood eosinophilia with myalgia (Greek: *mys*, muscle + *algos*, pain) emerged. With respect to Eosinophilia-Myalgia Syndrome (EMS) proper, reliance on a finite impurity in L-tryptophan-containing dietary supplements is both unnecessary and insufficient to explain the perplexing etiology. Excessive histamine activity has induced eosinophilia and myalgia. Termination of the multiple actions of histamine is dependent on particular amine oxidases and histamine-*N*-methyltransferase. Histamine metabolism is rapid when these degradative reactions are operative. The latent effects of incurred histamine can be potentiated and aggravating when these mechanisms are impaired. Overloads of tryptophan supplements cause – among other relevant side-effects – an increased formation of formate and indolyl metabolites, several of which, e.g., serotonin and tryptamine, inhibit the degradation of histamine. Endothelial cell lesions in EMS patients caused occlusive vascular disease, whereas serotonin and histamine can act as “capillary poisons” [Majno & Palade, J Biophys Biochem Cytol (1961) II: 571-605]. Moreover, (non-EMS) subjects with hypothalamic-pituitary-adrenal axis dysregulation have manifested greatly increased sensitivities to incurred tryptophan and histamine. In essence, a wide spectrum of primary data converges at the etiologic junction where compromised histamine degradation, abnormal eosinophil counts and myopathy overlap — whereby histamine disequilibrium appears to be a final common pathway for syndromes characterized by eosinophilia with myalgia [Smith & Garrett, Inflamm Res (2005) 54: 435-450].

P 21

Music therapy, "adverse" diet and histamine

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Music therapy is used to support psychological, physical and mental health. The wide spectrum of emotions, caused by music, is familiar to everyone. The great variety of sensations reaches from sympathy, joy and excitement to sadness and melancholy. The question arises as to whether music can also influence eating habits and histamine responses.

A group of 12 volunteers (students 19 – 43 years, 2 males) were tested by using a simple experimental design. The test persons were exposed to "good feeling" music while eating "adverse" (individual aversion to special food like uncooked egg and fish) test food. Anamneses and interview reports served as a basic reference assessment. Blood was drawn from the arm vein before and not later than 5 min after challenges. Histamine was measured using ELISA (Beckman-Coulter) and evaluated using fluorimetry after HPLC separation.

The majority of the tested volunteers showed significant responses to eating adverse diets ($p<0.05$, $n = 12$; Student's t-test): Pulse rates were elevated from 80 ± 6 to 94 ± 8 min^{-1} and histamine levels increased from 0.3 ± 0.2 to 0.85 ± 0.3 ng/ml during eating. When eating took place while listening to "good feeling" music the pulse rates and histamine levels remained approximately at the base values.

In the future more experiments are necessary to establish whether simple autodiagnostic methods – e.g. the pulse test – are sufficient for the assessment of the responses in music therapy.

Analysis of Human Basophil Activation Feed Back Induced by Histamine and High Histamine Dilutions

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The two main targets described so far for the negative feed back inducd by histamine on basophils are H2 receptors and more recently organic cation transporter 3 (OCT3) described in murine basophils. The results related to the biological activity of high histamine dilutions (HHD) showed that the activity of HHD paralleled the activity of histamine 10-4M on ionic flux, measured by alcian blue staining, on CD63 and CD203c expression and IgE/RIgE clustering measured by flow cytometry, both for IgE dependant (allergen or anti-IgE) and IgE independent (fMLP) basophil activation. The magnitude of the effect depended on the activation marker used, in the increasing order : CD63, CD203c, IgE/RIgE and ionic flux (15 to 70%), the inhibition observed fo histamine 10-4 M being in the same order of magnitude for all these markers (70-80%). HHD were less active on anaphylactic degranulation (AND measured by CD63) than on piecemeal degranulation (PMD) or membrane events. Histamine induced negative feed back was modulated by diamine oxydase (DAO), cimetidine, IL3 and lithium ion. Pre-incubation of histamine dilutions with DAO (0.1U/ml) reversed the activity of histamine 10-4M and had no effect on HHD (ionic flux). Cimetidine 10-5M added to the activation buffer reversed the effect of histamine for both dilution ranges (ionic flux and CD63 expression), lithium used at a sub toxic concentration (10µg/ml), reversed the effect of HHD (not tested on histamine 10-4M) (CD63 expression) and hIL3 used at a sub activating concentration (2 ng/ml) reversed the effect in both cases (CD63 expression).

The activity of DAO may be due to a decrease of available exogenous histamine, HHD being unsensitive to the enzymatic activity, lithium may have an intracellular activity and/or an activity on hydrogen bounds (this latter being confirmed by thermoluminescence). In contrast, hIL3 and cimetidine have identified potential targets, respectively RIL3 and RH2, at least for histamine 10-4M. Interestingly, histamine induced feed back was reverted at both dilutions ranges suggesting similar inhibition pathways.

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Nonlinearity in the Dose-response Curve of Extremely Diluted Histamine

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Withdrawn

P 24

Influence of Morphine- or Apomorphine-Induced Sensitization on Histamine State-Dependent Learning in the Step-Down Passive Avoidance Test

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Withdrawn

P 25

Excitatory Effect of Histamine on Neuronal Activity of Rat *Globus Pallidus* by Activation of H_2 Receptors *in Vitro*

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Previous studies have revealed distribution of histaminergic fibers and histamine receptors in the globus pallidus (GP). In this study, the rat brain slice preparation was used to examine the effect of histamine on the spontaneous unitary discharge of GP neurons. Within 95 extracellularly recorded GP neurons, 87 (91.6%) were excited by histamine. The histamine-induced excitation was concentration-dependent and persisted in low Ca^{2+}/high Mg^{2+} medium (*n* = 9), demonstrating that the action of histamine on GP neurons was postsynaptic. The excitatory effect of histamine on GP neurons was not blocked by selective H_1 receptor antagonist triprolidine (*n* = 16) or chlorpheniramine (*n* = 6), but was effectively suppressed by ranitidine, a selective H_2 receptor antagonist (*n* = 21). Selective H_2 receptor agonist dimaprit mimicked the excitatory effect of histamine on GP neurons (*n* = 23), while H_1 receptor agonists, including 2-pyridylethylamine (*n* = 22), 2-thiazolyethylamine (*n* = 9) and betahistine (*n* = 9), did not cause GP neurons any response. The dimaprit-induced GP neuronal excitation was effectively antagonized by ranitidine (*n* = 14) instead of triprolidine (*n* = 12). Moreover, adenylate cyclase (AC) activator forskolin (*n* = 7) was observed to evoke GP neurons an excitatory response, whereas the histamine-induced excitation was effectively reduced by H-89 (*n* = 9), a selective and potent inhibitor of protein kinase A (PK_A). Finally, it was noted that neurons in both subdivisions of the GP, the medial (*n* = 35) and lateral (*n* = 60) segments, showed no differences in their responses to stimulations of the tested histaminergic reagents. These results demonstrated that histamine excited GP neurons via H_2 receptors and H_2 receptors linked intracellular G-protein-AC-PK_A signaling pathway, suggesting that the hypothalamic histaminergic afferent fibers innervating GP may play an important modulatory role in motor control through its excitatory effect on GP neurons.

P 26

The Effects of Histaminergic Agents in the Dorsal Hippocampus of Rats in the Elevated Plus-Maze Test of Anxiety

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Withdrawn

P 27

Histamine Improves Rat Rota-Rod and Balance Beam Performances through H_2 Receptors in the Cerebellar Interpositus Nucleus

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Previous studies have revealed a direct histaminergic projection from the tuberomamillary nucleus of hypothalamus to the cerebellum and observed a postsynaptic excitatory effect of histamine on the cerebellar interpositus nucleus (IN) neurons via histamine H_2 receptors *in vitro* (Shen, Li & Wang, Brain Res, 948:64-71, 2002). Therefore, the physiological function of histamine receptors in the cerebellum and whether the histaminergic afferent inputs of deep cerebellar nuclei are involved in the cerebellar function of motor control were particularly interested in. In this study, histaminergic agents were bilaterally microinjected into the cerebellar IN of intact adult male rats, and their effects on motor balance and coordination of the animals performing accelerating rota-rod treadmill and balance beam tasks were observed. The results showed that microinjection of histamine into the cerebellar IN remarkably increased the time that animals balanced steadily on the rota-rod and markedly shortened the duration of passage through the balance beam, whereas □-aminobutyric acid significantly depressed motor performances of animals on the rota-rod and beam, and normal saline influenced neither. In addition, administration of selective histamine H_2 receptor antagonist ranitidine considerably decreased the animals' endurance time on rota-rod and noticeably increased the passing time on beam, but selective histamine H_1 receptor antagonist triprolidine showed no effect. Furthermore, microinjection of histamine reversed the inhibitory effects of ranitidine on rota-rod and beam performances. These results demonstrate that histamine enhances rat motor balance and coordination through activation of histamine H_2 receptors in the cerebellar IN and suggest that endogenous histamine released from the hypothalamocerebellar histaminergic projection terminals may play a modulatory role on the cerebellar circuitry to ensure movements to be accurately executed.

P2Y Receptor Mediated Excitation in the Posterior Hypothalamus

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Histaminergic neurons located in the posterior hypothalamus (tuberomamillary nucleus, TMN) project widely through the whole brain and control arousal and attention. They are tonically active during wakefulness but cease firing during sleep. As a homeostatic theory of sleep involves ATP depletion and adenosine accumulation in the brain, we investigated now the role of ATP and its analogues on neuronal activity in the TMN. We show increased firing of rat TMN neurons by ATP, ADP, UTP and 2meSATP, indicating activation of receptors belonging to the P2Y family. Adenosine – a sleep promoting substance – did affect neither membrane potential nor firing of these cells. Single cell-RT-PCR revealed $P2Y_1$ and $P2Y_4$ expression in TMN neurons. $P2Y_1$ receptor mRNA was detected with a higher frequency in 2-week-old than in 4-week-old rats; in accordance, 2meSATP was more potent than ATP with a threshold concentration of 0.25 µM versus 5µM, respectively. Semi-quantitative real-time PCR revealed a developmental down-regulation of mRNA levels for $P2Y_1$ and $P2Y_4$ receptors. Immunocytochemistry demonstrated neuronal and glial localization of the $P2Y_1$ receptor protein. Network activity measured with multielectrode arrays (MEAs) in primary cultures made from the posterior hypothalamus was enhanced by UTP and 2meSATP ($P2Y_4$ and $P2Y_1$ agonists, respectively). ATP caused an inhibition of firing, which was reversed in the presence of suramin or gabazine ($GABA_A$ receptor antagonist), indicating that GABAergic neurons are preferentially activated by ATP in this network. Excitation of the wake-active TMN neurons by nucleotides and the lack of adenosine action may be important factors in sleep-wake regulation.

P 29

Central Histaminergic System – the Regulatory Mechanism of Circulatory Homeostasis in Haemorrhagic Shock

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In this presentation, recent data concerning the role of endogenous central histamine in cardiovascular regulation in haemorrhagic shock are shown. A model of irreversible haemorrhagic shock by Guarini et al., in which rats were subjected to hypotension of 20-25 mmHg, leading to the death of all control animals within 30 min was employed. The increase in endogenous central histamine concentrations, which was achieved either by inhibition of histamine N-methyltransferase (HNMT) activity or loading with L-histidine, produced the reversal of critical hypotension and improvement of survival. HNMT inhibitor metoprine produced increases in mean arterial pressure (MAP), heart rate (HR) and peripheral blood flows. These were associated with rises in plasma noradrenaline, adrenaline, arginine vasopressin, angiotensin II, ACTH and α-MSH concentrations. Metoprine-induced changes in MAP and regional blood flows were reduced by α_1- and α_2-adrenoceptor antagonists prazosin and yohimbine, respectively, while β-adrenoceptor blocker propranolol diminished only a rise in HR. The resuscitating effect was also inhibited by V_{1a} receptor antagonist, V_{1b} and V_2 receptor blockers had no effect. Moreover, angiotensin type 1 receptor antagonist ZD 7155 and angiotensin-converting enzyme inhibitor captopril decreased regional vascular resistance and inhibited metoprine-induced increase in MAP. Finally, melanocortin type 4 receptor antagonist HS014, acting centrally, diminished MAP and regional vascular resistance changes. In conclusion, these results clearly demonstrate central histamine-induced activation of the sympathetic nervous system, the renin-angiotensin system and secretion of AVP and proopiomelanocortin-derived peptides during the resuscitation from haemorrhagic shock in rats. Thus, the strong evidence is provided for an involvement of the histaminergic system in the maintenance of circulatory homeostasis in hypotension.

Amitriptyline Affects Guinea Pig Post-Heparin Plasma Diamine Oxidase Activity

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Amitriptyline (AMI) is still widely used drug, indicated not only for the treatment of depression. Our previous studies have shown that antidepressants, including AMI affect pharmacokinetics of histamine in cat and rat. Diamine oxidase (DAO) is one of the most important histamine metabolizing enzymes, however, its amount in plasma is low but increases after heparin administration. The aim of present investigation is to establish whether AMI changes DAO activity in post-heparin plasma. Experiments were performed on male guinea pigs. Animals were pre-treated with AMI (ip.), followed by heparin (iv.) or with heparin (control). We took blood samples and measured DAO activity in plasma. Additionally in *in vitro* experiments guinea pig tissues (small intestine, lung, liver) and plasma were incubated with AMI (15 min) and/or heparin (30 min). Specific enzymatic activities of DAO were determined by radiometric assay procedure. Our results confirmed high post-heparin plasma DAO activity 5 min after the injection of heparin. In contrast, it was significantly lower in animals pretreated with AMI. AMI by itself did not alter plasma DAO *in vivo*. Heparin significantly released small intestine DAO in *in vitro* conditions, while in other tissues heparin had much less effect on DAO release. AMI decreased heparin-induced DAO release from the tissues, particularly from small intestine. It can be concluded that AMI diminishes heparin-induced DAO liberation from different tissues in *in vitro* and *in vivo* conditions. Since the majority of post-heparin plasma DAO is released from small intestine, we suspect that AMI can cause small intestine brush border damage or change and DAO could not be released after i.v. injection of heparin. Thus, when post-heparin plasma DAO is used as a biomarker for gastrointestinal epithelium diseases in animals or humans treated with AMI, we should take into consideration that AMI affects post-heparin plasma DAO activity.

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Immunolocalization of Histamine H_3 Receptor in the Rat Gastrointestinal Tract

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The histamine H_3 receptor (H_3R) is a presynaptic receptor, reported to be abundantly expressed in the central nervous system of different animal species while its presence in the periphery remains controversial. The aim of the present study was to explore the possible existence and location of the H_3R in the rat gastrointestinal tract using immunohistochemistry.

Groups of rats were treated with (R)-α-methylhistamine, 100 mg/kg, or saline, intragastrically 48h before sacrifice and half the rats in each group were fed *ad libitum* or fasted for 24h before sacrifice. Tissue samples were taken from stomach, small intestine and colon, fixed in 10% (v/v) formalin, prepared for immunohistochemistry and probed with our anti-H_3 349-358 antibody previously generated in rabbits (Chazot *et al.*, 2001) and validated using H_3R knockout mice (Cannon, Chazot *et al*. 2006)

Anti-H_3 receptor immunoreactivity (anti- H_3R IR) was differentially distributed in the various regions of the gastrointestinal tract. In the gastric fundus, anti-H_3R IR was almost exclusively found in the epithelial cells of the basal half of the mucosa. The number of positive cells per gland was 4.02±0.30 in fed rats and 3.03±0.27 in fasted rats. Cells immunopositive for histidine decarboxylase (HDC) paralleled in number and location those positive for the H_3 receptor. No anti- H_3R IR was detected in antral epithelium. Rare positive cells were present in the small intestinal epithelium, with a progressive decrease in their number from duodenum to distal ileum. No anti-H_3R IR was found in colonic epithelium. Occasional positive cells were found in the submucosa of the different regions examined. Prior treatment with (R)-α-methylhistamine did not influence the number of positive cells.

This is the first demonstration that the histamine H_3 receptor is expressed in the gastrointestinal mucosa, mainly in the gastric fundus and, to a lesser degree, in the duodenum.

Cannon K, Chazot PL, Hann V, Shenton FC, Hough LB, Rice F. J Comp Neurol 2006;*in press*

Chazot PL, Hann V, Wilson C, Lees G, Thompson CL. NeuroReport 2001;12:259-62

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P 32

Influence of H_3/H_4 Receptor Antagonist Thioperamide on Regional Haemodynamics in Rats with Trinitrobenzene Sulfonic Acid-Induced Colitis

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We have previously reported beneficial effects of thioperamide treatment in a rat model of inflammatory bowel disease. Here we present data on its influence on colonic haemodynamics.

Colitis was induced in ketamine/xylazine-anaesthetised rats by intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 25mg in 0.8ml 37% ethanol). The animals were injected daily with thioperamide (2mg/kg i.p.) or saline. Controls received 0.8 ml of saline. At 5 and 7 days after TNBS rats underwent a midline laparotomy under urethane anaesthesia for haemodynamic measurements. Inferior mesenteric artery blood flow (IMBF) was measured using ultrasonic transit time technique (Transit Time Flowmeter Type 700, electrode type 1RB2006, Hugo Sachs Elektronik, Germany). Macroscopic scoring of colonic damage was performed using a 10 point rating scale.Tissue and plasma histamine concentrations were measured by fluorimetric and radioenzymatic assays, respectively. There was increased IMBF at 5 and 7 day of colitis in both groups in comparison to the control. Interestingly, in thioperamide-treated rats, IMBF was significantly lower than in saline-treated animals at 7 day after induction of colitis (2.79±0.47 vs. 3.88±0.61ml/min). Macroscopic damage score was significantly increased at 5 and 7 day of colitis in comparison to the control. There were no significant changes in mucosal damage between the two groups 5 days after TNBS installation, however, at 7 day, the scoring in thioperamide-treated was significantly lower than in saline-injected rats (4.33 vs. 6.66, P=0.013). The colonic histamine concentrations were significantly decreased while plasma histamine levels were higher in TNBS-rats saline-treated compared to the thioperamide-treated ones. We conclude that histamine, acting via H3/H4 receptors, influences inferior mesenteric blood flow in TNBS-induced colitis. The study supports and extends our previous observations that thioperamide treatment accelerates healing processes in inflamed colon.

P 33

Effects of Histamine on Hepatic Segmental Vascular Resistances in Dog, Rabbit, Guinea pig, Rat and Mouse

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Withdrawn

P 34

Functional Gut Mucosal Biopsy Testing using Tolerated Food Antigens during Mucosa Oxygenation Yields a High Extent of Specificity in Non-Atopic, Non-Allergic Healthy Individuals

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The incidence of gastrointestinal food allergy has been reported to increase. This study examined the mediator response of normal gut mucosa from 6 healthy persons with complete oral tolerance towards all foodstuffs.

Living biopsies from normal colorectal mucosa were incubated during mucosa oxygenation (pO_2 90mmHg, pH 7.0, 37°C, INTESTINO-DIAGNOSTICS Erlangen) with various staple food antigens, which have been shown during food challenge tests to be tolerated from all individuals. The clinical score obtained by in vivo food antigen provocation was each lower than 4 points (no symptoms). Mediator release was followed over 4 hours by sequential measurement of histamine, tryptase (TRY) or ECP release [ng/mg wet weight, mean $\pm$ SD]. Antigenic stimulation of biopsies was compared with the spontaneous mediator release.

Normal colorectal mucosa showed a spontaneous release of histamine 0.47 $\pm$ 0.2, TRY 0.8 $\pm$ 0.5 and ECP 2.3 $\pm$ 2.3. This physiological mediator release was not significantly different from mediator release in response to tolerated food antigens. Histamine release in response to proven tolerated food antigens was 0.35 $\pm$ 0.4, TRY release 0.66 $\pm$ 0.3 and ECP release 1.7 $\pm$ 1.6, thus indicating that tolerated foodstuffs neither induced any mediator release nor false positive mast cell or eosinophil stimulation. This is further supported by the observation that no tolerated food antigen induced a stimulation index greater than 2 (mediator release food antigen in relation to spontaneous release) as shown for histamine 0.63 $\pm$ 0.6, TRY 0.81 $\pm$ 0.5 and ECP 0.82 $\pm$ 0.6.

Tolerated food antigens induced no pathological mast cell or eosinophil mediator release from normal colorectal mucosa. Furthermore, gastrointestinal tolerance was demonstrated by lack of any stimulatory effect of these foodstuffs, thus indicating that local mediator detection during mucosa oxygenation may identify food antigens which may be tolerated in certain diseases.

P 35

Mechanisms Underlying the Radioprotective Effect of Histamine on Small Intestine

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We have reported that histamine (HA) enhances radiosentivity of malignant cells and protects normal tissues from high doses of ionizing radiation (IR). HA reduces mucosal atrophy, edema and preserves villi, crypts and nuclear and cytoplasmic characters of small intestine after IR exposure. The aim of the present study was to investigate the mechanisms involved in HA radioprotective effect.

For this purpose, 36 mice were separated into 4 groups: Control (C), HA, irradiated (IR) and irradiated receiving HA (HA+IR). Irradiated animals received a single whole-body dose of 10 Gy. HA treated mice received a daily sc injection (0.1 mg/kg) during 5 days, starting 24 h before irradiation in the HA+IR group. All animals were sacrificed simultaneously after the experimental period. Antioxidant enzymes: superoxide dismutases (MnSOD and CuZnSOD), catalase (CAT) and glutathione peroxidase (Gpx) were determined by immunohistochemistry. In addition we evaluated HA content, expression of proliferating cell nuclear antigen (PCNA) and apoptosis by TUNEL assay.

Results indicated that C group expressed PCNA in crypts, MnSOD only in villi, CAT, CuZnSOD, Gpx and HA in both villi and crypts. The only change exerted by HA was the appearance of MnSOD expression in crypts. On the other hand IR produced a marked decrease in Gpx, the complete disappearance of PCNA expression and an increase in apoptosis. In contrast, in HA+IR group, HA completely reverted the effect of IR on PCNA expression, decreased the number of apoptotic cells and significantly increased CAT and CuZnSOD in both villi and crypts. As well as in HA group, MnSOD was present in crypts.

The data obtained clearly indicate that HA modulates the antioxidant enzymes, increases PCNA positive cells and inhibits apoptosis. Therefore HA may accelerate repair of damaged intestinal mucosae and/or increase proliferation and enhance antioxidant capacity, leading to the protection of small intestine against the cellular damage produced by IR.

P 36

Relative Therapeutic Efficacy of Tension Therapy and Histamine Iontophoresis in the Management of Indolent Ulcer- in a Sickle Cell Patient: a Clinical Report

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Withdrawn

P 37

Evaluation of Urinary N-methylhistamine Excretion During a Long-Term Follow Up of Patients with Inactive Crohn`s disease (CD)

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Mast cells are thought to participate in the pathogenesis of CD. Since urinary excretion of the histamine metabolite N-methylhistamine (UMH) is enhanced in active CD, the excretion of UMH was studied in patients with inactive CD (CDAI<150, no immunosuppressants) in order to evaluate if antihistamines could maintain remission. Methods: UMH was measured in 8 patients with inactive CD over a period of 1 year (6 measurements for each patient) and compared to control persons. Urine samples were collected during the night for 12 hours (6.00 pm – 6.00 am) under a normal diet. 1 N HCL was added to the urine samples to avoid bacterial histamine production. UMH was measured by tandem mass spectroscopy and specified in μg/mmol creatinine x m2 BSA.

Mean UMH of CD was found to be relatively low (5.2 ± 2.5), was within the normal reference range of controls (< 6.5, n > 100 individulas) and was no significantly different from control values (4.6 ± 1.9). Mean coefficient of variation in CD was 22.3% ± 8.4%. Individual means of UMH ranged between 2.6 ± 0.6 and 8.0 ± 1.2, whereas individual coefficients of variation were between 7.9% and 38.9%.

By comparison, Crohn`s disease activity index (CDAI) values represented a substantially higher variability with a mean coefficient of variation of 51.5% ± 38.3% than UMH. Interestingly, UMH showed a higher positive correlation with CRP ($r = 0.39$, $p = 0.07$) than CRP and CDAI ($r = 0.24$, $p = 0.1$), whereas an inverse correlation was found between UMH and CDAI ($r = –0.41$, $p = 0.01$).

In patients with inactive CD the urinary excretion of N-methyl-histamine lies in between the reference range compared to the enhanced UMH excretion in patients with active CD. In addition, UMH values demonstrate lower intraindividual variations than CDAI values. As a result, it is not promising to apply antihistamines in order to maintain remission. Nevertheless, UMH is an adequate parameter to indicate the activity of an acute episode of CD.

P 38

Second-Generation Antihistamines (SGAs): A Systematic Review of their Effect on Quality-of-Life (QoL) in Intermittent Allergic Rhinitis (IAR)

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SGAs are a mainstay of treatment for patients with IAR. A recent survey (Golightly LK & Greos LS, *Drugs* 2005;65:341-84) identified several clinical trials that acknowledged benefit in such patients. However, this survey failed to precisely define the level of benefit documented in these trials, particularly regarding treated patients' QoL.

This review sought to objectively assess the magnitude of change afforded by SGA treatment on QoL measures in patients with IAR.

Published studies of SGAs were identified through searches of the US National Library of Medicine's MedLine database. Studies were selected if they were recent randomized, placebo-controlled clinical trials of once-daily oral SGA therapy in adults with IAR graded moderate to severe in intensity using the 28-item Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ). Selected studies were included in a meta-analysis performed with Cochrane Collaboration *RevMan* 4.2 analytic software. Post-treatment mean and standard deviation RQLQ scores were incorporated into a random effects model and weighted mean differences (WMD) were determined.

Based on overall RQLQ scores, meta-analysis revealed a WMD of -0.44 (95% CI -0.43, -0.45; $p < 0.00001$) in favor of treatment. Although statistically significant, this falls short of the minimum important difference (MID) criterion of 0.5 that represents a clinically meaningful change.

SGAs were not distinguished from controls in terms of meaningful effect on QoL. Despite benefit proven in some individual trials, attainment of MIDs in QoL is not an effect common to SGAs as a class. These findings suggest that treatment of IAR should include preferential consideration of SGAs with higher potency or perhaps use of larger doses.

P 39

Do both Systemic and Topical Anti-Allergy Treatments Cause Ocular Dryness?

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Systemic antihistamines often have a side effect of ocular dryness, which is attributed to their M3 anticholinergic effect.

We wished to determine by retrospective analysis of pivotal trial data whether topical anti-allergy treatments also cause ocular dryness.

In a literature review, we compared the incidence of dry eye compared to placebo for the 3 most widely prescribed topical anti-allergy treatments. We also reviewed the results of trials involving loratadine and cetirizine relative to signs and symptoms of dry eye.

In the epinastine HCl 0.05% trial, the incidence of dry eye in the active group was 1/158 (0.6%) compared to 1/228 (0.4%) for the placebo group. In the ketotifen 0.25% trial, dry eyes occurred for 15/330 (4.5%) in the active group, compared to 7/165 (4.2%) in the placebo group. In the olopatadine 0.1% trial, incidence of dry eye was 1/189 (0.5%) for the active and 1/96 (1.0%) for the placebo group. In a second olopatadine study, the incidence of dry eyes was 1/267 (0.4%) for the active group and 1/138 (0.7%) for the placebo group. Compared to baseline in normal patients, use of loratadine led to a mean increase of 107% in keratitis, a mean increase of 133% in conjunctival staining, a mean decrease of 33.7% in TFBUT, and a mean increase of 24.8% in ocular discomfort. Compared to baseline, after using cetirizine HCl for 4 days, there was a mean increase of 60% in keratitis, a mean increase of 49.7% in conjunctival staining, and a mean decrease of 19.6% in TFBUT (P=0.05).

We know that systemic antihistamines cause signs and symptoms of dry eye to worsen, and can see that the incidence of dry eye following repeated dosing with 3 different topical anti-allergy treatments is less than or equal to that of placebo. To avoid ocular dryness, focused therapy with topical treatments is clearly superior to systemic antihistamines.

Use of rEV131 for Prevention of Anterior Chamber Inflammation in an Animal Model

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The potent histamine-binding molecule rEV131 is derived from a native protein secreted in the saliva of the Rhipicephalus appendiculatus tick. The feeding tick is thought to secrete the protein in order to suppress the host's early inflammatory response. In addition to binding histamine, rEV131 has been shown to exert anti-inflammatory effects including inhibition of TNF-alpha secretion and neutrophil recruitment. These mechanisms suggest that this protein may modulate trauma-based inflammation. The purpose of this study was to evaluate rEV131 for anti-inflammatory capabilities in an animal model of ocular inflammation.

In this 7 day study, Dutch Belted pigmented rabbits (≥ 1.6 kg each; N=24) were randomized to receive rEV131 0.25%, rEV131 0.125%, prednisolone 1.0% (positive control), or vehicle (PBS, negative control) in the right eye. QID dosing with assigned treatment occurred on study days 1, 2, 3. On Day 4, rabbits were dosed twice with assigned treatment, anaesthetized and subjected to laser irradiation in the treatment eye. Anterior chambers were examined for the presence of fibrin and other clinical signs of inflammation.

Prednisolone 1.0% and both concentrations of rEV131 demonstrated efficacy in preventing anterior chamber fibrin accumulation, compared to placebo. At peak time points, the efficacy of rEV131 0.25% appeared to exceed that of prednisolone 1.0%.

In this model, rEV131 demonstrated efficacy in preventing anterior chamber inflammation following trauma. This supports existing data suggesting that rEV131 exerts anti-inflammatory effects beyond binding histamine in acute allergic-based inflammation. Clinical efficacy of rEV131 may be comparable to that of steroids frequently used to treat post-cataract inflammation.

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Lidocaine Protein Binding Time Response to Diphenhydramine Administration in Rat Tissues

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Lidocaine, an amide, membrane stabilisator (pKa 7.6–8.0) possesses pharmacodymamic effect as anaesthetic even in low doses (4,4-5mg/Kg), useful in dental practice. In overdose it is accompanied by side effects from CNS; nausea, disorientation, paraesthesia, seizures and from CVS; disturbance of impulse conduction -cardiac arrest.

Diphenhydramine, an antihistamine first generation H_1 (pKa 9) has sedative, anticholinergic, antitussive, antivomiting and local anaesthetic activity. It is used as an alternative anaesthetic in clinical practice when the conventional ones induce allergic reactions or as a sedative agent in minor surgical operations.

The study's purpose was to investigate: a) the changes of lidocaine protein binding to serum and tissues proteins according to treatment time and b) the influence of diphenhydramine co-administration on the binding extent of lidocaine.

20 Wistar rats divided in 4 subgroups (n=5) I, II, III, IV were used. All groups received lidocaine infiltration in the masseter. Groups II and IV received diphenhydramine po 2h before the lidocaine injection. The protein binding of lidocaine was assessed by ultrafiltration in serum and tissue proteins with labeled H3-lidocaine. Specimens were obtained 15 and 30 min after lidocaine treatment with or without diphenhydramine. Statistical evaluation was performed *via* Wilcoxon test

Lidocaine's free fraction in serum and tissues was increased ($p<0,05$) in animals of group III (30 min) compared to group I (15 min). The co-administration of lidocaine and diphenhydramine resulted in an increase of the free fraction of lidocaine at 15 minutes, while at 30 minutes it was reduced.

The kinetics of lidocaine were altered by diphenhydramine either through the binding process or the elimination pathway from the tissues.

Xia Y, Chen E, Tibbits DL, Reilley TE, McSweeney TD. J Clin Anesth 2002;14:339-43

P 42

Locally Secreted Histamine May Regulate the Development of Ovarian Follicles by Apoptosis

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Histamine (HA) release within ovarian tissue during the estrus may correspond to cyclic changes of mast cells content and distribution in the ovary [1]. Most follicles in the ovary undergo atresia by apoptosis of somatic and germ cells. Pro-apoptotic effect of HA has been reported [2].

We examined influence of HA administration on the development of ovarian follicles in vitro with a special respect to apoptosis.

Ovarian preantral follicles (N=180) of 150-180μm isolated from immature mice were individually cultured in medium containing 5% immature mouse serum, supplemented with 100 mIU/ml recombinant human FSH, as method described in ref [3]. Diameter of the follicles was measured daily using x100 magnification and a calibrated micrometer. The effects of HA (10 mmol/l) and H1, H2 blockade (mepyramine and cimetidine, respectively) on apoptosis were examined daily in respective groups. The TUNEL method was used to assess apoptosis within ovarian follicles. Microscopic images were analysed in the ovarian sections using computed morphometry for quantitative analysis. At the end of culture period (5-6 days), follicles were induced to ovulate with hCG.

HA given in the preovulatory period significantly increased apoptosis (up to 196.08 ±17.4; % ±SD of intensity observed in controls in Day 3) and inhibited follicular growth, while administered in the culture matured for ovulation increased hCG-induced ovulation rate (30% versus 19% in controls; $p<0.05$) despite its pro-apoptotic effect. Histaminergic blockade canceled or significantly reduced the influence of HA on apoptosis, follicular growth and ovulation.

The results suggest that, depending on the developmental stage, HA acting as apoptosis inducer via H1and H2 receptors may play a role in the selection process of the dominant follicle, as well as may stimulate ovulation.

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P 43

Histamine H_4 Receptors in Human Placenta in Diabetes-Complicated Pregnancy

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The role of histamine in placental development has been described in many reports concerning physiological and complicated pregnancy. The effect of histamine action is mainly associated with histamine pro-angiogenetic potency. The main source of histamine in placenta are mast cells, but histamine may be released from trophoblast cells as well. It seems that histamine influences trophoblast development via H_1 receptor, but the other receptors may be involved as well.

In pre-gestational diabetes mellitus (PGDM) it is observed that placental vascular network is significantly increased, while gestational diabetes mellitus (GDM) does not influence angiogenesis in placenta. The effect of histamine on placental angiogenesis in PGDM is likely to be complex as the expression of histamine receptors may be regulated by many factors like cytokines or oxygen concentration.

The aim of the study was to evaluate expression of histamine H4 receptor in normal placentae and in placentae from pregnancies complicated by different types of PGDM.

Placental samples from normal pregnancies (control group) and pregnancies complicated by PGDM (classified according to White as: class B, C, D and F/R) were obtained after delivery and fixed. Immunohistochemical stainings with specific anti-H4R antibodies were used to evaluate the expression of histamine H4 receptor protein. Morphometric analysis were performed to assess the quantity and intensity of color reaction.

Differences in localization of color reaction were observed between particular classes of PGDM. Intensity of color reaction was different in control group and PGDM group.

It is the first report concerning presence of histamine H4 receptor protein in placenta. The results suggest that through this receptor histamine may be involved in controlling the placental function.

Histamine Increase the Invasive Potential of Human Melanoma Cells

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The incidence of malignant melanoma was significantly increased recent years. The patients with the disease exhibit reduced life expectancy. Histamine is an important paracrine and autocrine regulator of normal and tumor cell proliferation, as well. Melanoma cells reveal autonomous histamine metabolism.

Our aims were to demonstrate that histamine plays a key role in the invasion of melanoma cells. We investigated the effects of histamine on the migration, expression of adhesion molecules, chemokines, matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) in two human melanoma cell lines with different invasive potential.

Modified Boyden-chamber technique was applied to evaluate the effects of histamine on the migration. Immunocytochemistry, flow cytometry and real-time RT-PCR were carried out to estimate correctly the changes of the expression following histamine treatments with different concentration and administration time.

We found that histamine had chemorepellent effect on both melanoma cell lines. Expression of CXCR-4, CXCL-12, MMP-2, MMP-3 and VEGF were upregulated, while CD44, NCAM and ICAM-1 were downregulated upon the histamine treatments. Histamine plays a key role in several steps of the metastatic cascade such as migration, adhesion and invasion.

Our *in vitro* results suggest that histamine decreases directly the migration ability of human melanoma cells. On the other hand, histamine indirectly increases the invasive potential of melanoma cells by upregulating CXCR-4, CXCL-12, MMP-2, MMP-3 and VEGF, and by downregulating adhesion molecules.

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Endogenous and Exogenous Histamine Affects on Growth of Mice Mammary Adenocarcinoma

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Solid tumors require neovascularization for their growth. Here, we investigated the effects of histamine (HA) on tumor growth and angiogenesis. Syngeneic mammary adenocarcinoma cells, (LM2) were inoculated to the flank of BALB/c mice. Mice were injected daily with either saline or HA. As a second model for investigating the role of endogenous HA in regulating immunosuppression, angiogenesis and tumor growth, we used the transgenic HDC knockout BALB/c mouse strain that has a targeted disruption in the histidine-decarboxylase gene coding for histamine synthesis. Tumor size was measured daily. A complementary DNA (cDNA) array with 96 genes that modulate angiogenesis was used to identify differentially expressed genes (2-fold higher or lower) in tumors. Real-time quantitative polymerase chain reaction was used to confirm cDNA array expression data.

HA markedly enhanced tumor growth, the highest growth rate was shown in the wild-type mice. Eight genes were upregulated in HA treated mice by cDNA array analysis, but only 4 genes had higher messenger RNA (mRNA) expression levels by real-time quantitative polymerase chain reaction. Exogenous HA inhibited the expression of Eph receptor B1 (EphB1) and ephrin-ligand B1 (EfnB1).

Angiopoietin-2 and-4 (ANGPT2 and ANGPT4) and EfnA2 mRNA expression was elevated, IL-12 , IL-1 and IL-18 was reduced in wild –type mice compared with HDC KO ones. Our findings suggest that HA promotes tumor growth, at least in part, by enhancing tumor-associated angiogenesis. Ligands for receptor tyrosine kinases (RTKs) have emerged as critical mediators of angiogenesis. Three families of ligands, vascular endothelial cell growth factors (VEGFs), angiopoietins and ephrins, act via RTKs expressed in endothelial cells. In this study, we provide evidence that EfnA2 and ANGPT4 are necessary for induction of neoangiogenesis and they are regulated by HA in this model.

Nitric Oxide Involvement in Histamine-mediated PANC-1 Cells Growth

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Panc-1 cells overexpress H1 and H2 histamine receptors (H1R, H2R). Histamine (HA) in concentrations higher than 1 μM activates the adenylate cyclase signaling pathway via H2R and inhibits cell proliferation. It has been reported that nitric oxide (NO) exerts antiproliferative action in different tumoral cell lines. Moreover, HA induces the nitric oxide synthase (NOS) expression and increases NO production in endothelial cells.

The aim of this work was to study HA-induced NOS modulation and its possible involvement in PANC-1 cell growth.

Cell proliferation was evaluated by the clonogenic method. A dose-dependent inhibition on cell growth was observed when cell cultures were treated with NOS inhibitors (L-NAME, EC50: 1.5 ± 0.5 nM; Aminoguanidine, EC50: 250 ± 30 μM) and the NO donnor (SIN-1, EC50: 10 ± 3 μM).

Endothelial and inducible NOS (eNOS and iNOS) isoforms expression was determined by RT-PCR. Data indicated that PANC-1 cells express constitutive eNOS and that it is positively modulated at 24 h by 10 μM HA, 10 μM Forskolin (Fk, adenylate cyclase direct activator) and 4 mM L-NAME. Conversely, a decrease in eNOS mRNA levels was observed when cells were treated with 20 μM SIN-1. iNOS mRNA expression was undetectable when tested at the previous mentioned experimental conditions. A significant augmentation of intracellular NO level was demonstrated by flow cytometric analysis employing the fluorescent dye DAF-2DA after cell cultures were exposed to 10 μM HA (140 ± 13%), Fk (162 ± 18%) or L-NAME (153 ± 15 %).

In conclusion, NO levels modulate eNOS expression and cell growth in PANC-1 cells. Results also indicate that the inhibitory effect exerted by HA on cell proliferation may be mediated by HA-produced NO levels.

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Differential Modulation of the Cellular Stress Response by Histamine and the H_1, H_2 Receptor Antagonists in Eukaryotic Cells

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The cellular stress response is a universal inducible reaction of cells exposed to adverse environmental/microenvironmental conditions, such as heat or drugs, associated in part with the highly conserved heat shock proteins (hsp's). Inflammatory reactions related to histamine (HI), like the stress response, are crucial for cell survival, while HI is known to interact with hsp's in mammals. Its metabolizing enzyme systems are evolutionary conserved in eukaryotes, including the yeast, an established model for the pharmacological investigation of the cellular stress response. This study aimed at exploring the possible role of HI and H_1, H_2 receptor (H_1R, H_2R) antagonists on the stress response in yeast. The response was evaluated by determining microscopically the viability of post-logarithmic phase grown cell cultures after heat shock (HS) at 53 ^oC for 30min. The effect of HI, dimetindene maleate and ranitidine was investigated following preconditioning with the agents for 2h prior to the HS. The induction of tolerance by thermal preconditioning of yeast cultures at 37°C for 2h served as a positive control. The expression of hsp's was determined by western blotting. Administration of either HI or the H_1R antagonist dimetindene maleate resulted in a dose-dependent increase of viability, contrary to the H_2R antagonist ranitidine, which showed no effect. The presence of cycloheximide, a *de novo* protein synthesis inhibitor, led to a significant decrease in viability after HI preconditioning but did not alter the effect of the H_1R antagonist. No morphological cellular alterations were observed upon addition of the agents. Preliminary investigation of hsp104, hsp70 and hsp60 expression showed an increase of these proteins after thermal preconditioning. However, a differential expression of hsp's appeared to underlie the response of yeast cells to histamine and the H_1R/H_2R antagonists.

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Effects of Histamine *via* H_4 Receptor on Splenic Dendritic Cells

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Withdrawn

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Histamine H_1, H_2 but not H_4 Receptors are Upregulated during Bone Marrow Regeneration

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Earlier it was found that after total body irradiation bone marrow regeneration was delayed in histidine decarboxylase knockout (HDC-ko) mice compared to wild type (WT) mice. In the present study the role of histamine receptors behind this difference was investigated.

HDC-ko and WT mice kept on histamine free diet were subjected to 4 Gy whole body gamma-irradiation. Bone marrow samples were obtained in the 6th hour and on the 1st, 3rd and 7th days. H1R, H2R, H4R and HDC mRNA levels were determined by real-time PCR. HDC and histamine content of immunophenotypically characterised (CD34+, Sca1+, Ckit+, STR, LTR) cell populations of WT mice were measured using flow cytometry.

The mRNA expression of H1R and H2R was increased in the 6th hour and on day 1 after irradiation in both HDC-ko and WT mice. While after the 1st day these levels decreased to the control value in HDC-ko mice, in WT mice mRNA expression remained at a high level throughout the regeneration phase. However, H4R mRNA expression was downregulated in both groups. mRNA of HDC was increased from the 6th hour after irradiation. Elevated HDC enzyme content of various WT bone marrow cell populations was detected on the 1st day after treatment, and it remained at a high level during regeneration. Mainly CD34+ cells were responsible for this elevation. However, histamine content of these cells was increased already after the 6th hour. While high concentrations of histamine were measured in CD34+ cells on all studied days, in the earlier (CD34-) hematopoietic progenitors it soon returned to the control level after the initial increase.

The increase in both H1R and H2R but not in H4R expression levels and also the elevated histamine content of hematopoietic progenitors support the role of histamine in bone marrow regeneration. The increase in the HDC content of these progenitor cells proves the regulatory role of their endogenous histamine synthesis in hematopoietic differentiation processes.

Unexpected Action of Histamine on Dendritic Cells under Acidosis

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We have shown that acidosis improves uptake of antigens and MCH class-I restricted presentation by dendritic cells (DC). Here we analyzed whether acidosis may be able to modulate the regulatory action exerted by histamine (H) on DC. DC were obtained from C57BL/6 bone marrow progenitors cultured with GM-CSF. They were cultured at pH 7.3 or 6.5 during 1 h, washed and suspended at pH 7.3, and treated with H (1 μM) for 30 min at 37°C. Then, we evaluated the uptake of OVA-FITC and the production of IL-12 by flow cytometry. H increased endocytosis by DC and this effect was improved in pH 6.5-treated DCs: pH 7.3, 27±2,3; pH 7.3 + H, 53±4,8; pH 6.5, 64±5,9; pH 6.5 + H, 126±11,2 (mean fluorescence intensity ±SE, n=7, $p<0,05$). Despite the well known ability of H to suppress the production of IL-12, we found that under acidosis H increased the production of IL-12 by DC: % of positive cells for IL-12 = pH 7.3, 0.5 ± 0.3; pH 7.3 + H, 0.6 ± 0.3; pH 6.5, 7.1 ± 1.4, and pH 6.5 + H, 15 ± 0.6. We finally examined whether acidosis could modify the normal expression of the two major H receptors expressed in DC, which usually mediate opposite effects. RIA binding assays showed that exposure of DC to acidosis resulted in a two-fold increase in the expression of H2R and a reduction of 75% in the expression of H1R. Our results suggest that extracellular acidosis may be able to subvert the regulatory action exerted by H on DC.

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Tissue-Related Immune-Endocrine Interactions in Experimental Hyperthyroidism

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Inflammation and inflammatory responses are modulated by bidirectional communication between the neuroendocrine and immune systems. Cytokines released from activated immune cells can act in the brain to elicit a range of centrally mediated responses. Furthermore, the function of the immune system is influenced by circulating hormones and transmitters. Our study sought to investigate the possible involvement of immune mediators in the integration of the histaminergic response in hyperthyroid rats. Male Wistar rats of 300-350g bw were divided into 3 groups of 5-7 animals each. Experimental hyperthyroidism was induced in group 1 by s.c. injection of 0.25mg/Kg T_4 qdx10d. Group 2 was injected s.c. with 30mg/Kg sodium cromoglycate (SCG) qdx10d, 45min prior to T_4 administration. Group 3 received saline and served as control. All animals were sacrificed 24h after the last dose. Following sacrifice, the hypothalami and the cartilaginous part of the 9th and 10th right costosternal junction were obtained. Histamine (HI) was quantified fluorometrically and IL6, IL2, IL4, IL10 and TNFα were determined by ELISA. The results were expressed as the mean±SEM of the % of their respective controls. Statistical analyses were performed by ANOVA and one sample test. In hyperthyroid rats, HI was significantly increased in the cartilage compared to control, in contrast to the decreased HI levels in the hypothalamus. The same pattern was observed for IL2. In hyperthyroid rats, administration of SCG failed to alter the HI content in cartilage, while it restored the amine levels in the hypothalamus. Moreover, SCG did not influence IL2 levels, but tended to decrease the hypothalamic IL10 and IL6 content. Preliminary data showed no alteration of TNFα levels upon any treatment. In conclusion, these data implied a diverse state of the immune components in different tissues, under experimental hyperthyroidism conditions.

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Histamine Stimulates Electrogenic Ion Transport in Avian Epithelia

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Although histamine is recognised as a secretagogue in mammalian intestine, relatively little is known of its action upon epithelial ion transport in avian tissues. The objectives of this study were two-fold; i) to characterize actions of exogenous and endogenous histamine on ion transport across intestinal epithelia from the domestic chicken *Gallus Gallus* and ii) to determine histamine release from the same tissues in response to compound 48/80.

Birds were euthanased and sheets of ileal mucosa were harvested, stripped of underlying smooth muscle, and mounted in Ussing chambers. Tissues were maintained at 37^{o}C and bathed on either side with identical volumes of bicarbonate-buffered physiological solution which was aerated and mixed using a 95%O_2/5%CO_2 uplift system. Spontaneous electrical potential differences across isolated epithelia were voltage clamped to zero by the continuous application of a short-circuit current (SCC). Drugs were added either to the basolateral or to the lumenal compartments. In separate experiments, histamine release from tissues was determined by ELISA.

Exogenous histamine evoked a rapid onset inward SCC (EC_{50}=6.5 M) which was accounted for by chloride secretion since it was attenuated by bumetanide or by chloride-free bathing solutions. Histamine was more effective when added to the basolateral domain than when added lumenally. Compound 48/80 produce SCC responses which were quantitatively and qualitatively similar to those of exogenous histamine. Mepyramine (1 M), which had no effect on ion transport responses to carbachol, virtually abolished SCC responses to histamine and to compound 48/80. Responses to compound 48/80 were also attenuated by the mast cell stabilising agent ketotifen (1 M).

In conclusion, these data suggest that endogenous histamine released from a mast cell-like avian cell results in H_1-receptor mediated chloride secretion. *In vivo*, consequent fluid secretion may be a mechanism for expulsion of lumenal pathogens or toxins.

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Peripheral but not Central Effects of Allergic Reaction are Mediated by Histamine

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Systemic allergic reaction as a significant threat to homeostasis stimulates neurosecretory neurons in the hypothalamic paraventricular nucleus (PVN) triggering activation of the hypothalamo-pituitary-adrenal axis. Histamine released from mast cells plays a well-defined role in the course of allergic symptoms, however little is known about its role in mediating central effects of allergic challenge. In our recent experiment we assessed the influence of H1-receptor signalling on cellular and transcriptional changes in the stress-related neurocircuitry provoked by anaphylactoid reaction. To provoke anaphylactoid reaction, adult male Wistar rats received an intraperitoneal injection of ovalbumin. Brains were perfusion fixed two hours later, at the time point that is known for maximal level of challenge-induced cFos protein and corticotropin-releasing hormone (CRH) mRNA in the PVN. cFos protein, the marker of neuronal activation was revealed by immunocytochemistry, CRH mRNA as a marker of activation of the stress-related circuit was visualized by isotopic in situ hybridization. The number of cFos positive profiles significantly increased ($p=0.009$) in all the three functionally distinct subdivision of PVN, and the CRH mRNA level was also elevated in the medial parvocellular PVN ($p=0.003$). To study the involvement of H1-receptor system, two different doses of H1-antagonist pyrilamine (5 and 10 mg/kg) was intravenously administered. Both doses of pyrilamine effectively attenuated peripheral signs of anaphylactoid reaction. In contrast to the peripheral effects of the H1-receptor antagonist, pyrilamine failed to prevent cFos induction ($p=0.3$) and transcriptional activation of CRH gene ($p=0.9$) in the PVN. Our results support the involvement of histamine through H1-receptors in the development of peripheral symptoms of allergic reactions; however other mediators and/or receptor systems may contribute to immune-to-brain communication during systemic anaphylactoid challenge.

Study of Adipogenesis in Genetically Histamine Free (HDC KO) and Wild Type Bone Marrow Derived Mesenchymal Stem Cells

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The role of histamine in allergic reaction is known for decades, however, its role in cellular differentiation less well studied.

Bone marrow derived mesenchymal stem cells (MSCs) of histidine decarboxylase deficient (HDC KO) and wild type (WT) mice were used to study the involvement of histamine in differentiation processes. As a first step we excluded the presence of hematopoietic stem cells by FACS in both of our mesenchymal stem cell cultures. The number of colony forming units (CFUs) was significantly reduced in HDC KO cultures. Based on previously published data showing a mild late onset obesity in HDC KO mice, we studied the adipogenic potential of MSCs. Increased adipogenesis was observed in the differentiated HDC KO mesenchymal stem cells after Oil Red staining.

Expression of histamine receptors (H1R, H2R, H3R, H4R), Oct4 (a stem cell specific marker), CREB and Gsk3 (both involved in adipogenesis) were analysed by real time PCR in differentiated and undifferentiated MSC cultures. H3R, H4R and Oct4 were not expressed in none of the studied samples. On the other hand there was a four-fold increase in CREB and Gsk3 expression in undifferentiated KO cultures, while their mRNA levels were reduced after differentiation. H1R and H2R were evenly expressed in undifferentiated cells of both genotypes, however, following differentiation only the transcription of H2R was increased.

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Internalization and Resensitization Mechanisms of the Histamine Type 2 Receptor

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We have previously reported that the magnitude and duration of H2R agonist-evoked response are key features in biological processes such as cellular differentiation *(Biochem. Biophys. Res. Commun., 314:798, 2004).* Thus, in COS-7 cells the termination of the signal is dependent upon GRK2 and GRK3 induced phosphorylation of the H2R *(Mol. Pharmacol., 60:1049, 2001)*. Once a GPCR is phosphorylated it may suffer either internalization and degradation or recycling to the membrane following dephosphorylation. Further receptor dephosphorylation may occur without internalization. Given the relevance of such mechanisms in the desensitization/resensitization process of receptor-evoked responses, we sought to establish H2R trafficking following selective agonist treatment. Confocal microscopy and binding assays performed in H2R transfected COS-7 cells showed that the number of H2R sites in the membrane was reduced by 50% after 60 min agonist stimulation but it was fully recovered 60 min after stimuli withdrawal. Furthermore, cAMP accumulation evoked by H2R stimulation with a selective agonist for 60 min was reduced by 90% but it was partially recovered (50%) when the stimulus was removed. Arrestin and dynamin are two important proteins involved in GPCRs internalization. Cotransfection studies with negative dominants for these two proteins showed that although the number of H2R sites in the membrane remained unaltered following agonist stimulation, the receptor response resulted abolished and failed to recover after the stimulus was removed. Present findings support that although internalization is not necessary for H2R desensitization it is relevant for active sites recycling to the membrane. In addition results show that arrestin and dynamin are involved in H2R endocytosis and recycling.

Strain Specific Differences in Oct4 Expression of HDC KO and Wild Type ES Cells

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Although there is a plethora of information about the functions of histamine in different physiological processes, little is known about its function in cell diffrentiation and development. In order to study these events ES (embryonic stem) cells proved to be good candidates. Therefore we established ES cell lines from WT and HDC (histidine decarboxylase) KO mice with two different genetic backgrounds. Our experiments were carried out on both CD1 and Balb/C mice derived WT and HDC KO ES cells.

According to our earlier observations expression of the stem cell specific marker, Oct4 changed during the cardiac differentiation, and at least during the later stages of in vitro differentiation it was modified after histamine treatment.

It seemed to be reasonable to analyse whether these alterations are dependent only on the presence of histamine.

When we measured the expression of Oct4 by real time PCR we found that in genetically histamine free ES cells – independently from their origin – Oct4 expression was dramatically reduced. On the other hand histamine, in the applied concentrations, could modify Oct4 expression only in CD1 derived ES cells. When we analysed H1R and H2R expressions under the same conditions an even stronger strain specific difference was observed: only Balb/C derived WT ES cells showed receptor upregulations upon adding histamine.

Possibly histamine is not directly involved in Oct4 regulation, but there are other still unidentified players in its regulatory pathway.

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Comparison of the *in Vitro* and *in Vivo* H_2-H_4 Receptor Selectivity of 4-Methylhistamine and VUF8430

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The identification of potent and selective histamine H_4 receptor ligands is mandatory to unravel the biological function of the H_4 receptor [1]. Previously, we have reported on the *in vitro* and *in vivo* H_4 receptor effects of various H_2 agonists and identified 4-methylhistamine as potent H_4 receptor agonist [2]. Further research resulted in a potent H_4 receptor full agonist VUF8430 with a pK_i value of 10 nM. In the present study we have compared VUF8430, 4-methylhistamine and the related H_2 agonists dimaprit and amthamine for their H_2 and H_4 receptor activities both *in vitro* and *in vivo*. *In vitro*, VUF8430 shows the largest ratio for the H2/H4 receptor binding affinities and acted as a full H_4 receptor agonist. In the anaesthetised rat with lumen-perfused stomach, intravenous (iv) administrations of 4-methylhistamine (10 mg/kg), dimaprit (30 mg/kg) or amthamine (3 mg/kg) produced similar increases of basal acid secretion (4.18 ± 0.85, 4.56 ± 0.35 and 5.21 ± 0.8 μEqHCl/kg/min, respectively). VUF8430 was ineffective at 10 mg/kg iv and induced a weak acid secretory effect (1.74 ± 0.34 μEqHCl/kg/min) at 30 mg/kg iv. VUF8430 and 4-methylhistamine administered at 10 mg/kg sc in conscious rats did not damage the gastric mucosa *per se* and induced a non significant aggravation of gastric lesions induced by indomethacin 20 mg/kg sc. In inflammation models, VUF8430 (10 mg/kg sc) or 4-methylhistamine (10 mg/kg sc) did not significantly modify the paw edema induced in conscious rats by subplantar injections (0.1 ml) of carrageenan (1% in carboxymethylcellulose). In conclusion, VUF8430 is a new highly potent, full agonist at the H_4 receptor and displays a large margin of selectivity with respect to H_2 receptor activation.

[1] de Esch IJ et al. Trends Pharmacol Sci 2005;26:462-9
[2] Lim H et al. J Pharmacol Exp Ther 2005;314:1310-21

Porcine Plasma Amine Oxidase has a Broad Substrate Specificity and Efficiently Converts Histamine

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Blood plasma contains considerable amine oxidizing activity. In most mammals, the enzyme responsible for the major part of this activity is the product of the AOC4 gene, a soluble copper-containing amine oxidase produced and secreted primarily by the liver. To assess the possible function of this enzyme especially with respect to histamine inactivation we purified the amine oxidase enzyme from porcine plasma and studied its substrate specificity and kinetic properties. From 25 ml plasma approximately 5 mg homogenous protein were obtained in five consecutive chromatographic steps with 64% yield and 325-fold enrichment. The purified protein is a glycosylated homodimer with an apparent molecular weight of 210 kDa. Purified porcine plasma amine oxidase is stable and remains active for months when stored frozen. Radiometric and luminometric activity assays revealed a broad substrate specificity for this enzyme. It efficiently converts aromatic and aliphatic amines but also polyamines and histamine. K_m values indicate a preference for aromatic amines and longer polyamines whereas short-chain aliphatic mono- and diamines have a lower binding affinity. These enzymatic properties suggest that the main function of plasma amine oxidase is the inactivation of all sorts of amines in the circulation to limit their biological half-lifes. Although diamine oxidase (DAO), the product of the AOC1 gene, has been implicated as the primary histamine oxidizing enzyme, plasma amine oxidase may be important for the inactivation of histamine in plasma where DAO is normally not present. Humans do not possess a functional AOC4 gene and might therefore be more sensitive to the effects of plasma histamine that has escaped the action of DAO at the initial site of release.

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Influence of Amitriptyline on Central Histamine-induced Reversal of Haemorrhagic Shock in Rats

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Exogenous histamine administered intracerebroventricularly (icv), similarly to endogenous histamine after loading with histamine precursor L-histidine or inhibition of histamine N-methyltransferase activity, leads to the reversal of critical haemorrhagic hypotension in rats. The effect is due to activation of the sympathetic nervous system and the renin-angiotensin system and secretion of arginine vasopressin and proopiomelanocortin-derived peptides. On the other hand, our previous studies demonstrate that tricyclic antidepressant amitriptyline (AMI), acting centrally and peripherally, is able to affect vascular resistance regulatory mechanisms in intravenous histamine-induced hypotension in rats. Thus, the aim of the present study was to examine a possible influence of AMI on central histamine-evoked resuscitating effect in haemorrhage-shocked rats. Experiments were carried out in ethylurethane-anaesthetised male Wistar rats. We used a model of irreversible haemorrhagic shock by Guarini et al., in which animals were subjected to hypotension of 20-25 mmHg, leading to the death of all control rats within 30 min. The bleeding volume for induction of critical hypotension was 2.23 ± 0.2 ml/100 g body weight. Histamine (50 nmol; icv) administered to haemorrhage-shocked rats evoked long-lasting rises in mean arterial pressure (MAP) and heart rate (HR), with a 100% survival of 2 h. Pre-treatment with AMI (4 nmol; icv) increased central histamine-induced rises in MAP and HR, with no influence on survival, whereas AMI (4 nmol; icv) given alone had no effect. In contrast, AMI at higher doses (8, 20 nmol; icv) evoked short-lasting rises in MAP, with no influence on survival in comparison to the control group. In conclusion, AMI affects the reactivity of the cardiovascular centre to hypotensive stimuli and increases central histamine-induced resuscitating effect. The action can be related to inhibition of noradrenaline and serotonin re-uptake.

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Altered Inflammatory Gene Expression in Genetically H_4 Histamine Receptor Deficient Mice

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Histamine is a well-characterized inflammatory mediator acting through a variety of receptors. H4 histamine receptor (H4R) is expressed in different immune cell types, which potentially may modify the innate immune response, including inflammation (de Esch IJ, Thurmond RL. Trends Pharmacol Sci. 2005;26:462-9.). The role of this receptor in the acute phase reaction has not been studied yet. The systemic acute phase reaction (APR) is the part of non-antigen specific immune response and is a consequence of the inflammation. One of the characteristic features of APR is the alteration of plasma protein gene expression in the liver.

The aim of this study was to reveal the alteration of hepatic acute phase reactant basal and bacterial (E. coli) lipopolisacharide induced gene expression in genetically H4R deficient (SV129) mice and revealing the systemic alterations of the major inflammatory cytokines involved in the regulation of hepatic APR.

cDNAs were developed from total liver RNA. Hepatic gene expression was detected by semiquantitative PCR. Blood cytokines were quantified with commercial ELISA kits.

More then two times higher basal haptoglobin, transferrin and α_2-macroglubulin mRNA expressions were found in the H4R deficient animals. No significant differences were observed in the mRNA levels of serum amyloid A and complement C3. Serum IL-6 was increased both in the non-treated and LPS injected H4R knock out mice.

The elevation of serum IL-6 explains the over-expression of haptoglobin, transferrin and α_2-macroglobulin well. The difference in serum IL-6 level was very consistent, but the mechanism by which H4R deficiency leads to this alteration requires further study.

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Involvement of the Histaminergic System in the Central Cardiovascular Regulation in Haemorrhage-Shocked Rats with Portocaval Anastomosis; Preliminary Data

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As it was shown earlier, the increase in endogenous central histamine concentrations, following inhibition of histamine N-methyltransferase activity or loading with histamine precursor L-histidine, leads to a long-lasting H_1 receptor-dependent resuscitating effect in haemorrhage-shocked rats. Rats with portocaval anastomosis (PCA), an animal model of hepatic encephalopathy, demonstrate very high neuronal histamine content due to an excessive brain neurotransmitter formation after the shunt. Therefore, in the present study, we examined the role of central histamine in cardiovascular regulation in haemorrhage-shocked PCA rats. Experiments were carried out in ethylurethane-anaesthetised male Lewis rats subjected to a haemorrhagic hypotension of 20-25 mmHg for 15-20 min. Volumes of blood necessary to induce critical hypotension in PCA rats and in the sham-operated control ones were 1.34 ± 0.37 and 2.12 ± 0.16 ml/100 g body weight ($P < 0.05$), respectively. In the control group, the animals demonstrated no changes in mean arterial pressure (MAP) and heart rate (HR), and died within 30 min of shock. In contrast, there was a spontaneous recovery from critical hypotension, with increases in MAP and HR, and a 100% survival rate of 2 h in rats with PCA. Changes in MAP and HR in haemorrhage-shocked PCA rats were in part reduced by histamine H_1 receptor antagonist chlorpheniramine (100 nmol), administered intracerebroventricularly at the end of bleeding period. We conclude that the central histaminergic system is involved in spontaneous recovery from hypotension in PCA rats, and histamine action is mediated by H_1 receptors. We suggest a possible participation of other mechanisms in the spontaneous reversal of haemorrhagic hypotension in PCA rats, for example large amounts of residual blood, existence of PCA and changes in other central neurotransmitter systems. However, regulatory mechanisms responsible for the maintenance of circulatory homeostasis during haemorrhage are less sufficient in rats with PCA.

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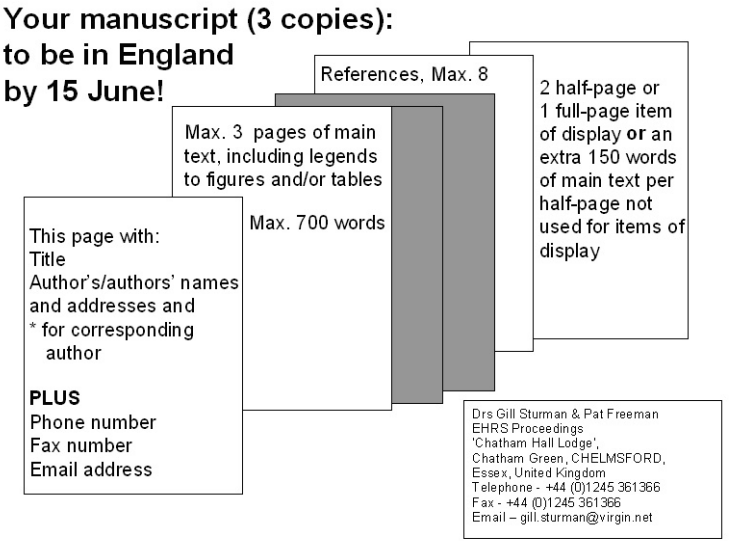

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If 2 tables or 1 table and 1 figure	up to 10 lines (not longer than 85 characters) and no more than 10 columns

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INFLAMMATION RESEARCH publishes papers on all aspects of inflammation and related fields including histopathology, immunological mechanisms, gene expression, mediators, experimental models, clinical investigations and the effects of drugs. Related fields are broadly defined and include for instance, allergy and asthma, shock, pain, joint damage, skin disease as well as clinical trials of relevant drugs.

In addition, the Journal publishes meeting reports, letters, opinions, news of people, policies and general information relating to the above fields and the societies active in these fields.

The types of papers published are original research papers, short communications, reviews, commentaries, selected reviewed society proceedings and meeting reports.

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

References In the text, references must be given as consecutive numbers in square brackets. Wherever possible, excessive citation should be avoided. The reference list should be typed on a separate sheet(s) at the end of the manuscript, numbered according to consecutive citation in the text, using the Vancouver system as follows:

Heaney LG, Cross LJM, Stanford CF, Ennis M. Substance P induces histamine release from human pulmonary mast cells. Clin Exp Allergy 1995;25:179-86.

Skidmore IF, Vardy CJ. The mediators of bronchial asthma and the mechanism of their release. In Saxena PR, Elliot GR, eds. Pathophysiology and Treatment of Asthma and Arthritis. Agents and Actions Suppl. vol. 14. Basel: Birkhäuser, 1984:33-48.

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

For further examples see: N Engl J Med 1991;324:424-8.

Journal abbreviations should correspond to those in the World List of Scientific Periodical. Where possible, references should be easy to find.

A paper which has been accepted for publication but which has not yet appeared may be cited in the reference list with the words "in press" after the abbreviated name of the journal.

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

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

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

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

diameter, preferably chosen from: ● ¦ ▲ ○ □

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

Legends should be typed on a separate sheet(s) of paper paginated as part of the paper.

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

Submission of manuscripts

Please send the original and 2 paper copies of your manuscript together with a copy on disc.

Texts on disc should be delivered in IBM PC format. They should be saved and delivered in two separate versions: with standard format as offered by your word processing program (give details of the program and version on the disc) and in Rich Text Format.

Hard copy originals of illustrations must be 200% of printed size.

Digital drawings, graphs etc should be submitted in JPG, GIF or TIF.

...about DELPHI

The temple ofApollo in Delphi

DELPHI
Past and Present

Delphi (Δελφοί) is a World Heritage site, located in the mountainous district of Central Greece (Sterea Ellada), at an altitude of 600m on the slopes of Mount Parnassos, NE of the olive tree valley that stretches to the Corinthian Gulf. It is passed by the major road linking Athens, ca. 180 km SE, with Amfissa, the capital of the Prefecture of Fokis, ca. 15 km SW.

According to the myth, Dias (Zeus, Jupiter) released two eagles, one from the east and the other from the west. At the point where they met, he threw the sacred stone to locate the geographical centre of the world; the navel (Gk: omphalos) of the earth. It was at that point that the sanctuary of Delphi was developed. Blending harmoniously with the superb landscape, Delphi was antiquity's most important centre of worship and a symbol of unity of the ancient Greek world.

Delphi, then called Pytho, a rocky place in Homer's Iliad, was inhabited in prehistoric times by the goddess Gaia (Earth). The development of the sanctuary and oracle began in the 8th century B.C. with the establishment of the cult of Apollo, who taught people the most ethical and peaceful expression of the Greek spirit: *the metron* (prudence and modesty; nothing in excess). In the Homeric Hymn, Apollo arrived from Delos; he killed the chthonic serpent Python and he took over the Oracle (Pythian Apollo), thus introducing the worship of Delphinios Apollo at Krisa (today Hrisso). The area owns its name, possibly to Apollo who appeared in the form of a dolphin (Gk: delphis, dolphin; womb, distinguishing the dolphin as a mammal or indicating the archaic veneration of Gaia). Another legend relates Apollo to St. George. Apollo shot his arrow from the *Katoptireon*, over the nearby ski resort of Arahova, to kill the dragon in order to leave the water running free for the people. Interestingly, in the Christian ages, St. George killed the Dragon for the same reason and he is worshipped by the Arahovians to this day.

Under the direction of the 12 Greek tribes of the Amphictyonic League, the Pythian Games and Delphic Festivals were held every 4 years, in the 3rd year of the Olympiad. In 191 B.C. the Romans became masters of Delphi and despite attempts to revive the oracle, it ceased to be regarded as the navel of the world. With the spread of Christianity, the sanctuary lost its significance and was closed down with a decree of the emperor Theodosius the Great in 392 A.D.

Inhabited since the distant past, Delphi is a pole of attraction for thousands of tourists who do not restrict themselves to the inexhaustible historical reference points. The area offers natural beauty, varied scenery and highly evolved tourism facilities. Still a remarkable location, Delphi is a headquarters for international meetings, cultural symposia and congresses held in the European Cultural Centre of Delphi that aims at reviving Delphi as a European and international cultural centre with global activities.

APOLLO (Απόλλων)

The Connection to Art, Science & Medicine

Apollo; detail from a coin c.336 B.C.

Apollo was one of the 12 gods and goddesses of the Olympian Pantheon (gods had *ichor* in place of blood, drank *nectar* and ate *ambrosia*). According to Hesiodos, he was the son of the supreme ruler Dias and the Titaness Leto, born with his twin sister Artemis (Diana) on the island of Delos. The Apollonian Hymn narrates that Dias' jealous wife Hera refused to allow Leto to give birth on any ground. Floating Delos was the only place willing to accept the birth of the powerful god and Leto anchored the island to the Aegean to show her appreciation.

Apollo enjoyed eternal youth and good health and represented harmony, order and reason. He shared the shrine (on his leave in autumn) with Dionysus, the god of wine, representing emotion and chaos, in contrast or complementary -according to the Greeks- to Apollo. Temples were raised in his honour throughout the ancient world, the most famous being those in Delos and Delphi and the Asclepion on the isle of Kos, where Hippocrates founded the western world's first medical school.

The Hippocratic Oath is an oath to Apollo the Physician, his son Asclepios and his "granddaughter" Hygeia, the goddess of Health. The serpents of Asclepios wrapped around his staff and the bowl of Hygeia have become the symbols of medicine and pharmacy in modern times, respectively. Asclepios' connection to the dual, therapeutic and toxic, pharmacological effects of drugs is illustrated by the properties of the magic potion given to him by the goddess Athena. The potion was from the blood of the Gorgon and if taken from the right side, it could bring the dead back to life, but taken from the left it was a deadly poison!

In addition to healing, Apollo was also the god of light, arts, music, poetry, archery and prophecy. His most famous attributes were the cithara, the lyre, the laurel crown and the tripod, the symbol of his prophetic powers. At Parnassus, Apollo was the leader of the Muses and he was associated with laurel after the story of his rape of Daphne (Gk. for laurel or bay-tree). Being the god of divination, he taught Cassandra the art of prophecy, but after deceiving him, she was unable to convince anyone with her prophecies!

The spirit expressed 30 centuries ago in Delphi still inspires art and science. The leader of the Muses has been immortalized throughout the centuries by countless poets, writers, painters, musicians and philosophers. In 1894, Baron Pierre de Coubertin had the first Delphic Hymn to Apollo performed at the Sorbonne to promote the idea of reviving the Olympic Games. Under the name of Phoevos (radiant or beaming), Apollo, together with Athena were designated as mascots of the 2004 Summer Olympics in Athens. Finally, one of the major missions of humankind, "Project Apollo" was named after the god of the Delphic oracle and aimed at landing a man on the Moon and return him safely to Earth. This goal was achieved with the Apollo 11 mission in July 1969.

Pythia on a tripod; c.440 B.C.

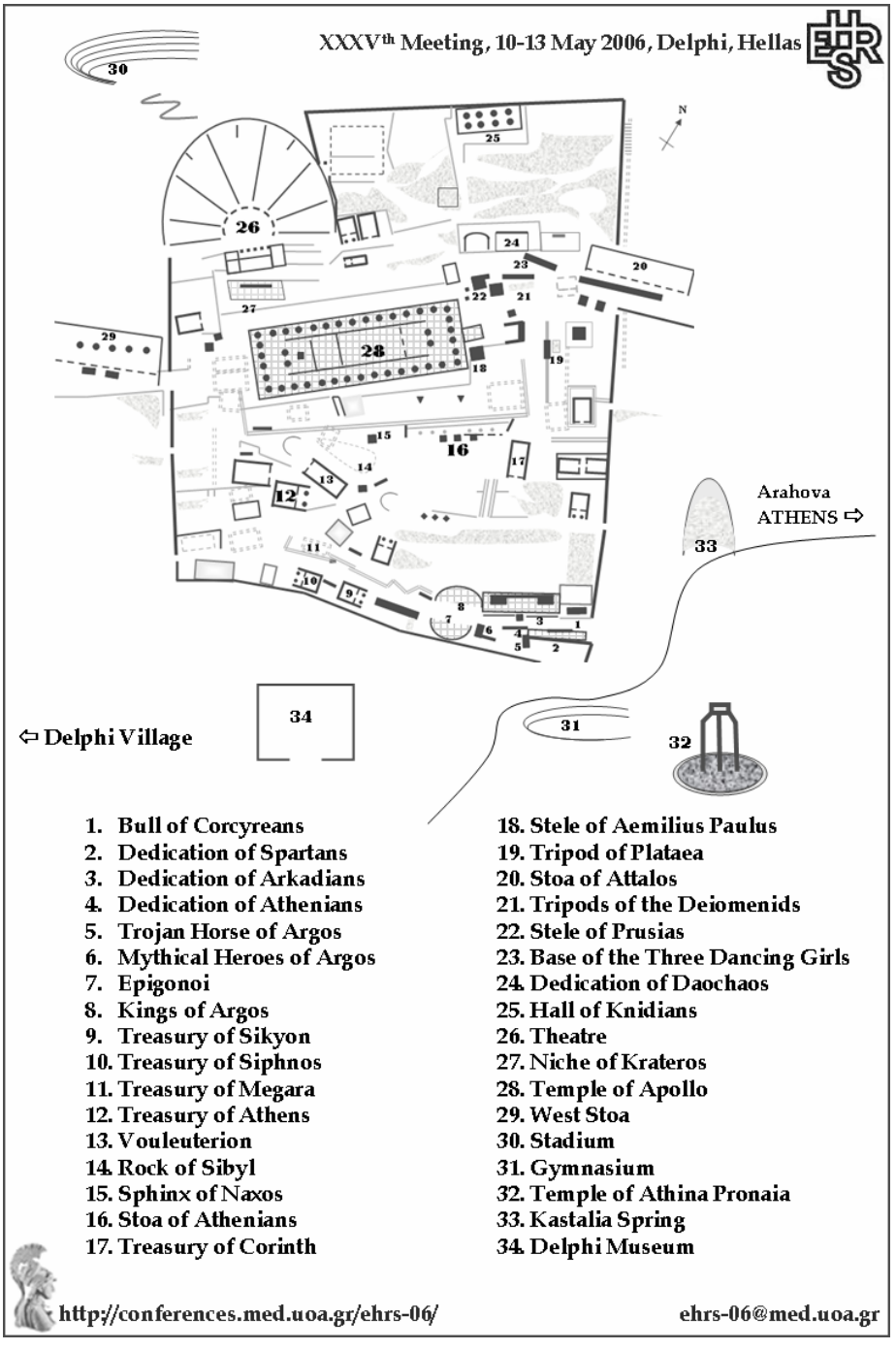
The Prophecy Ceremony (Chrismodotisis)

Visitors from all over the ancient world paid the tax (the pelanon) in order to be given the right to approach the altar to offer sacrifices and to seek advice from the oracle. On a fixed day each month, except in winter, Pythia purified herself in the sacred spring of Kastalia, entered the *adyton*, sat on Apollo's tripod and responded by bending over omphalos, inhaling hallucinating vapours. She entered a state of ecstasy and uttered ambiguous prophecies, which were then interpreted by the Priests of Apollo. Heraclitus said that Pythia never gave a straight answer. *The oracle neither concealed nor revealed the truth, but only hinted at it*.

The prophetic power of the Delphic oracle was attributed to a fissure in the bedrock, a gaseous vapour and a spring. Recent research provided evidence that the intoxicating gaseous emission was not a myth. The site of the oracle shows young geological faults intersecting below the temple, possibly emitting in ancient times light hydrocarbon gases from bituminous limestone, which have intoxicating effects. The pharmacological effects of ethylene inhalation match the well-documented effects of the ancient prophetic vapours.

The Archaeological Site

The excavations of the French Archaeological School began in the 1890's and revealed more than 5000 exquisite works of art. The *Museum* (34), being one of the richest in the world, contains findings from the site, including the navel of the world, the bronze Charioteer (Iniohos), metopes from the restored 6th century B.C. Doric *Treasury of Athens* (12). One can admire the 5th century B.C. 7000 spectators *Stadium* (30) that hosted the Pythian Games; the partly restored 4th century B.C. Doric *Temple of Apollo* (28), where Pythia delivered the oracular responses; the Ionic single-stone column *stoa of the Athenians* (16); treasuries and dedications of kings and cities of antiquity (1-25); the *Gymnasium* (31), where the youths of Delphi were educated; the sacred *Kastalia Spring* in the ravine of the Phaedriades (33), with two fountains dating to the Archaic period and the Roman era; the c.380 B.C. Doric Tholos of *Athena Pronaia* (32), a true architectural masterpiece of antiquity; the 5000 seat *Theatre* (26) that hosted the Delphic Festivals. It was in this theatre, that the poet A. Sikelianos and his wife, E. Palmer revived the Delphic Festival in the 1930's, wishing to "… *instil the forgotten Delphic watchword in all human souls*". Their house still stands in Delphi. At Livadi, close to the nearby ski resort of Arachova lies Koryceo Antro, a prehistoric cave-temple dedicated to god Pan.


XXXVth Meeting, 10-13 May 2006, Delphi, Hellas
N
Arahova
ATHENS ⇨
⇦ Delphi Village
1. Bull of Corcyreans
2. Dedication of Spartans
3. Dedication of Arkadians
4. Dedication of Athenians
5. Trojan Horse of Argos
6. Mythical Heroes of Argos
7. Epigonoi
8. Kings of Argos
9. Treasury of Sikyon
10. Treasury of Siphnos
11. Treasury of Megara
12. Treasury of Athens
13. Vouleuterion
14. Rock of Sibyl
15. Sphinx of Naxos
16. Stoa of Athenians
17. Treasury of Corinth
18. Stele of Aemilius Paulus
19. Tripod of Plataea
20. Stoa of Attalos
21. Tripods of the Deiomenids
22. Stele of Prusias
23. Base of the Three Dancing Girls
24. Dedication of Daochaos
25. Hall of Knidians
26. Theatre
27. Niche of Krateros
28. Temple of Apollo
29. West Stoa
30. Stadium
31. Gymnasium
32. Temple of Athina Pronaia
33. Kastalia Spring
34. Delphi Museum
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Δ Ε Λ Φ Ι Κ Ο Σ Υ Μ Ν Ο Σ

[Ἴ]τ΄ επί τηλέσκοπον τάαν(δ)ε Πα[ρνασί]αν
[φιλόχαρον] δικόρυφον κλεειτύν, ύμνωων
κ[ατάρ]χ[ετε δ΄ έμων], Πιερίδες, αινιφοβόλους
πέτρας ναι εθ΄[Ελι]κωνίδ[ας]
μέλπετε δέ Πύθιον χ[ρ]υσοχαίταν,
έ[κατ]ον, ευλύραν Φοίβον, όν
έτικτε Λατώ μάκαιρα πα[ραλίμναι]
κλυτάι, χερσί γλαυκα[ά]ς ελαίας
θιγου[ού-σ΄ όζον έν αγωνίαι]ς
εριθα[λή]

DELPHIC HYMN TO APOLLO

(translated by Richard Hooker)

Oh, come now, Muses, and go to the craggy sacred place
upon the far-seen, twin-peaked Parnassus,
celebrated and dear to us, Pierian maidens
repose on the snow-clad mountain top;
celebrate the Pythian Lord with the golden sword,
Phoebus, whom Leto bore unassisted on the Delian rock
surrounded by silvery olives, the luxuriant plant
which the Goddess Pallas long ago brought forth

HYMNE DELPHIQUE À APOLLON

Venez sur cette double cime regarde au loin, le Parnasse
Ami des chœurs, et présidez à mes chants, ô Piérides,
qui habitez les roches neigeuses de l' Hélicon
venez chantez le Pythien aux cheveux d' or,
le maître de l' arc et de la lyre, Phébus, qu' enfanta
l' heureuse Latone près du lac illustre,
quand, dans ses douleurs, elle eût touché de ses mains un
rameau verboyant de l' olivier

THE INTERNATIONAL ANTHEM OF THE EHRS

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!

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.

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

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

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

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

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

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.

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

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

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.

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

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

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.

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.

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

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

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

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

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.

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.

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

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

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

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

INDEX OF AUTHORS