

European Histamine Research Society XXXIX meeting Durham, UK 13th-16th July 2010



Programme and Abstracts

Photo by
Paul Sidney

Organiser: School of Biological & Biomedical Sciences, Durham University

Venue: Hatfield College and Elvet Riverside, Durham University



Sponsors



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BMBS



Participating countries: AT, CH, DE, DK, ES, FI, FR, GR, HU, IE, IL, IT, NL, PL, SE, SI, UK

Chair of the Action: Ekaterini Tiligada, GR, aitylliga@med.uoa.gr

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www.histamineresearch.com

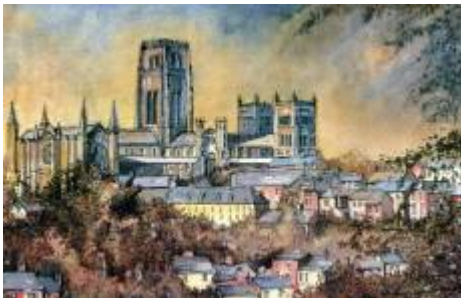


Welcome to Durham city and Durham University

World-renowned for its Christian heritage, Durham's history can be traced back to the arrival in the year 985 of a community of monks seeking a permanent resting place for the body of St Cuthbert, the founder of Christianity in Britain.

The name Durham means "hill on an island". It comes from the old English words "dun" meaning "hill" and "holmr" meaning island. A church was built for the monks and to house the body of Saint Cuthbert which continues to this day to act as a magnet for visitors from all over the world. Soon a town grew up on the site. It was an ideal site for a town as it was easy to defend and it had a major 'tourist attraction'.

In 1072 the Normans built a castle in Durham to keep the inhabitants in order. In 1083 they founded a Benedictine priory (a small abbey) to replace the community who looked after Cuthbert's body. In 1093 the Norman bishop of Durham, William of Calais began a cathedral. Cuthbert's body was finally laid to rest there in 1104. Durham Cathedral was completed in 1133. Several years later the building of the Cathedral began which was to provide a monumental shrine for the saint as well as a place of worship. Durham Cathedral has been described as 'one of the great architectural experiences of



Europe'. It is renowned as a masterpiece of Romanesque (or Norman) architecture. It is the only cathedral in England to retain almost all of its Norman craftsmanship, and one of few to preserve the unity and integrity of its original design.

Nearly one thousand years on, in 1832, Bishop Van Mildert and the Cathedral Chapter resolved to establish an 'Academic Institution or College or University' and their benevolence enabled the foundation of Durham University.

Durham University was founded as Britain's third university in 1832. The bishop gave the castle to the university to use as a college in 1837. Hatfield College was the second college to be established in 1846. The University has reason to be grateful for St Cuthbert for without the need to house a shrine to the saint, the Cathedral would not stand today and without the Cathedral, the University would not exist and the EHRS would not have a conference venue. Links between the Cathedral and Durham University remain strong to this day. Every year Durham welcomes thousands of pilgrims who come to honour St Cuthbert, explore the city and discover what makes it so special. One thing is certain; whether a staunch believer or a resolute agnostic, visitor or inhabitant of the city, nobody can fail to be stunned by the panoramic views of Durham Cathedral or recognise that the city's history is firmly rooted in its Christian heritage.

I hope you enjoy the Science, the City and the British weather....

Paul

<http://www.durhamcathedral.co.uk/history>

<http://www.dur.ac.uk/durham.first/summer07/christianheritage/>



Previous EHS Annual Meetings

1971 Lodz	1980 Visegrad	1990 Kuopio	2000 Nemi (Rome)
1972 Paris	1981 Hannover	1991 Marburg	2001 Turku
1973 Marburg	1982 Bled	1992 Malaga	2002 Eger
1974 Copenhagen	1983 Brighton	1993 Cologne	2003 Noordwijkerhout
1975 Florence	1984 Florence	1994 Budapest	2004 Bergisch-Gladbach
1976 Paris	1985 Aachen	1995 Moscow	2005 Bled
1977 London	1986 Odense	1996 Antwerp	2006 Delphi
1978 Lodz	1987 Strbske Pleso	1997 Seville	2007 Florence
1979 Stockholm	1988 Copenhagen	1998 Lodz	2008 Stockholm
	1989 Breda	1999 Lyon	2009 Fulda



Committees

Chairman of the meeting

Paul L Chazot

Organizing committee

Paul L Chazot

Anita Sydbom

Madeleine Ennis

Abstract Evaluation Committee

Anita Sydbom (Chairman)

Frank Ahrens

Patrizio Blandina

Paul Chazot

Marlon Cowart

Elena Rivera

Hubert Schwelberger

Gill Sturman

Bursary Committee

Anita Sydbom (chairman)

Frank Ahrens

Patrizio Blandina

Marlon Cowart

Elena Rivera

Hubert Schwelberger

Gill Sturman

The EHRS Young Investigator Award Committee

Holger Stark (chairman)

Beatrice Passani

Ekaterini Tiligada

Poster Prize Committee

Ekaterini Tiligada (chairman)

Konstantinos Papamichael

Jian-Sheng Lin

Christelle Anaclet

Emanuela Masini

Leonardo Munari

Peter Peachell

Tünde Simon





Scientific Programme

Oral sessions will take place in Debating Chamber, Palace Green

Poster sessions will take place in Hatfield College



Tues 13th July

10.00-16.00 **COST Action BM0806 Training School (WG1) for ESRs (Elvet Riverside 141, Palace Green)**

András Falus – Genomics & Bioinformatics

Paul L Chazot – Antibody production & validation

Abdel Ennaceur – *In vivo* preclinical studies

Emanuela Masini – Inflammation models

Rob Leurs - Medicinal Chemistry & receptor modelling

15.00 onwards Registration (Hatfield College Bar area)

16.00-18.00 EHRS Council Meeting (Birley room, Hatfield College)

16.00-18.00 Independent Break out group time (TBA)

18.30-19.30 **COST Action BM0806 Public Lecture: Professor Jean-Charles Schwartz** (Calman Building, Durham University Science Park)

Histamine as a neurotransmitter in brain: from discovery to novel psychotropic drugs- A short history dedicated to the memory of Sir James Black (1924-2010)

19.30 onwards Welcome Party (Hatfield College Bar area)

Wednesday 14th July

8.30-9.30 Opening session **Elvet riverside 141**

Welcome from Durham University

Paul Chazot (Chairman of the Meeting) Conference Programme & COST Action BM0806

Anita Sydbom (President EHRS) Introductory remarks & EHRS Bursary distribution



9.30-10.30 GB West Lecture: **Professor Jean-Charles Schwartz** (Bioprojet, France)
Clinical applications of pitolisant (BF2.649), an inverse agonist at the H₃ receptor

10.30-10.45 Coffee Break

10.45 – 12.00 Oral session I (**O1-O5**): **COST ACTION BM0806 Histamine H₄ receptors**

Chairpersons: Elena Rivera & Maria Gschwandtner

10.45-11.00 O1 Protein Fragment Complementation: A New Way To Look At Human H₄ Receptor Oligomerization

Saskia Nijmeijer¹, Henry F. Vischer¹, Paul L Chazot², Martine J. Smit¹ and Rob Leurs¹

11.00-11.15 O2 H₄R ligands induce STAT downstream phosphoregulation

Cathleen Krieg, Violetta Hames, Jutta Haefner, Susanne Diel, Hannelore Borck, Friedhelm Diel

11.15-11.30 O3 Role of histamine H₄ receptor in murine dendritic cell functions

Tünde Simon, Valéria László, András Falus

11.30-11.45 O4 Human memory Th17 cells express a functional H₄ receptor.

Susanne Mommert, Brigitta Köther, Maria Gschwandtner, Ralf Gutzmer, Thomas Werfel.

11.45-12.00 O5 JNJ7777120 Compound: a Potential Candidate for Use as Radioprotector

Vanina A Medina(1,2), Diego Martinel Lamas(1), Maximo Croci(3), Juan P Prestifilippo(4), Eliana Carabajal(1), Juan C Elverdin(4), Rosa M Bergoc(1,2), Elena S Rivera(1)

12.00 – 13.00 Oral session II (**O6-O9**): **COST ACTION BM0806 - Histamine H₄ receptors**

Chairpersons: Ralf Gutzmer & Saskia Nijmeijer

12.00-12.15 O6 Impact of H₃/H₄ antagonists on IgE and IgE-regulatory cytokine's synthesis

Roman Khanferyan, Nadegda Milchenko, Yulia Dorofeeva



12.15-12.30 O7 Histamine H₄ Receptor activation on human 6-sulfo LacNAc-expressing dendritic cells downregulates their proinflammatory capacity

Maria Gschwandtner¹, Knut Schäkel², Thomas Werfel¹, Ralf Gutzmer¹.

12.30-12.45 O8 Anti-inflammatory effects of a selective histamine H₄R antagonist in a rat model of carrageenan-induced pleurisy.

E. Masini, R. Mastroianni, C. Lanzi, M.R. Quaranta, ¹Thurmond R.L., ²Pini A., ²Bani D.

12.45-13.00 O9 Histamine downregulates IL-27, another member of the IL-12 family, via H₂R and H₄R on human monocytes.

Ralf Gutzmer, Brigitta Köther, Maria Gschwandtner, Susanne Mommert, Thomas Werfel.

13.00-14.00 Lunch

14.00-15.00 Plenary Lecture: Professor Patrizio Blandina (Florence, Italy)

Functional implications of Histaminergic Neurons Heterogeneity

15.00 – 16.00 Oral session III (O10-O13) (El-Sayed Assem Session): **General Histamine topics** Introduction by Bernie Gibbs

Chairpersons: Bernie Gibbs & Linda Kay

15.00-15.15 O10 Differential Modulation of Human Basophil Function by Ambroxol and Related Secretolytic Analogues.

Bernhard F. Gibbs

15-15-15.30 O11 Heterogeneity in the responses of human lung mast cells to Stem Cell Factor

Jessica Wan, Linda J Kay, Suzanne Havard, Peter T Peachell



15.30-15.45 O12 Histamine Signaling And Metabolism In Solid Organ Transplantation

Hubert G. Schwelberger

15.45-16.00 O13 Increased Pyroptosis in Human Amniotic Epithelial Cells (HAEC) in Chorioamnionitis (CHA) May be Related to Histamine Acting via its H₂ Receptor.

Dariusz Szukiewicz^{1,2}, Witold Rongies³, Danuta Maslinska^{1,4}, Anna Bilka¹, Katarzyna Sawicka¹, Sławomir Maslinski¹

16.00-19.00 Main Poster session (incl. COST Action communications) /Refreshments

(Hatfield College Bar area)

18.30-19.30 COST WG1/2 meeting (Birley Room)

19.30 Reception for 20.00 Dinner Lumbley Castle

Thursday 15th July

8.30-9.30 Plenary Lecture: **Dr Nick Carruthers (J&J, USA)**

Translational approaches towards the identification of a histamine H₃ receptor antagonist and its' clinical evaluation for the symptomatic treatment of allergic rhinitis

9.30 – 11.00 Oral session IV (O14-O19): **Histamine in the Brain I**

Chairpersons: Pertti Panula & Natasha Lethbridge

9.30-9.45 O14 Evidence for a role of histamine in motivation-driving wakefulness, study using knock-out mouse models.

R.X. Guo¹, M. Zhang¹, C. Anaclet¹, Q.L. Yao¹, C. Buda¹, J.P. Sastre¹, H. Ohtsu², O.A.Sergeeva³, H.L. Haas³
and J.S. Lin¹



9.45-10.00 O15 The Histaminergic System Regulates Wakefulness Through a Hypothalamo-telencephalic System and Orexin Cells in Zebrafish

Maria Sundvik¹, Hisaaki Kudo¹, Stanislav Rozov¹, Yu-Chia Chen¹, Walter Schunack² and Pertti Panula¹.

10.00-10.15 O16 The Histaminergic system in Parkinson's Disease

Ling Shan^{1,2}, Chun-Qing Liu¹, Koen Bossers², Rawien Balesar², Joop J. Van Heerikhuize², Juan-Li Wu¹, Natasha Lethbridge³, Paul L. Chazot³, Ai-Min Bao^{1,2} and Dick F. Swaab²

10.15-10.30 O17 H₃ receptor-dependent regulation of conditioned reward by ethanol

Jenni Vanhanen, Saara Nuutinen, Pertti Panula

10.30-10.45 O18 Involvement of the brain histaminergic system in the melanocortin MC4 receptor agonist RO27-3225-induced resuscitating effect in haemorrhage-shocked rats – haemodynamic studies

Jerzy Jochem¹, Daniela Giuliani², Alessandra Ottani², Maria Galantucci², Mariusz Krawitowski¹, Luca Spaccapelo², Salvatore Guarini²

10.45-11.00 O19 Histaminergic stimulation of astrocytic NT-3 synthesis: Mediation via histamine H₁, H₂ and H₃ receptors

Damijana M. Juric, Tina Mele, Marija Čarman-Kržan

11.00-11.30 Coffee break / Poster session (incl. **COST** Action communications)

11.30-12.15 Oral session V (O20-O22): H₃ receptors in the periphery

Chairpersons: Gabriella Coruzzi Konstantinos Kyriakidis

11.30-11.45 O20 Involvement of histamine in the gastroprotection induced by ghrelin in the conscious rat

Gabriella Coruzzi¹, Rob Leurs², Holger Stark³ and Maristella Adami¹

11.45-12.00 O21 Histamine H₃, rather than H₄ receptors, participate in regional blood flow regulation in rat model of ulcerative colitis

Wiesława Agnieszka Fogel¹, Katarzyna. Kiec-Kononowicz², Barbara Skrzydło-Radomska³ and Jerzy Jochem⁴



12.00-12.15 O22 Effects of the H₃ Receptor Inverse Agonist GSK334429 on the Histamine Levels of Cartilage, Oesophagus and Peripheral Blood Vessels in Rats with Adjuvant Arthritis

Konstantinos Kyriakidis, Evangelia Zampeli, Konstantinos Papamichael, Ekaterini Tiligada

12.15-13.15 Plenary Lectures: Drs Jorge Brioni (Abbott, USA) "Discovery of Novel Agents for the Treatment of CNS Disorders" & Marlon Cowart (Abbott, USA) "Histamine H₃ antagonist properties and efficacy in CNS models"

13.15 Lunch

14.00 onwards Excursion (**Beamish**)/Independent Breakout groups

Dinner/Entertainment - Northumbria Pipes & History Talk **Hatfield College Refectory**

Friday 16th July

7.30-8.30 **COST Action WG4 meeting**

8.30-9.30 **COST Plenary Dr Steve Liu (Pfizer, UK) Histamine H₃ and H₄ receptors – therapeutic opportunities in airway and inflammatory diseases**

9.30-10.30 Oral session VI (**O23-O25**) **EHR Young Investigator Award Symposium**

Chairpersons: Holger Stark, Beatrice Passani, Ekaterini Tiligada

9.30-9.45 O23 Complementary and Synergistic Control of Wakefulness by Orexins and Histamine, Demonstrated Using a Double Knockout Mouse Model.

Christelle Anaclet¹, K Ouk¹, G. Guidon¹, C. Buda¹, J.P. Sastre¹, H. Ohtsu², M. Yanagisawa³, HL Haas⁴, P. Franco¹ and J.S. Lin¹

9.45-10.00 O24 Major human histamine H₃ receptor isoforms display pharmacological differences

Natasha L. Lethbridge [1], Andrew D Medhurst [2], Paul L Chazot [1]

10.00-10.15 O25 Histamine and Dopamine in Alcohol Addiction: Friends or Enemies?

Saara Nuutinen, Tiia Ojala, Jenni Vanhanen, Pertti Panula

10.30-11.00 Coffee break & final poster viewing (incl. **COST** communications)



11.00-12.00 Oral session VII (**O26-O29**) **Histamine in the Brain II**

Chairpersons: Patrizio Blandina & Peter van Ruitenbeek

11.00-11.15 O26 Oleylethanolamide and Brain Histamine Interact to Regulate Feeding Behaviour

Leonardo Munari¹, Nicoletta Galeotti¹, Hiroshi Ohtsu², Patrizio Blandina¹ and M. Beatrice Passani¹

11.15-11.30 O27 Effects Of Citalopram On Tail Suspension Test Require The Presence Of Neuronal Histamine

Leonardo Munari, Maria Beatrice Passani, Nicoletta Galeotti, Patrizia Giannoni¹, Hiroshi Ohtsu², Patrizio Blandina.

11.30-11.45 O28 Dopamine-induced arousal depends on the histaminergic system

Olga A. Sergeeva¹, Helmut L. Haas¹, Evgenij Yanovsky¹, Sha Li^{1,2} and Jian-Sheng Lin²

11.45-12.00 O29 Central H₁-receptor blockade increases sedation, but does not affect memory in healthy humans

Peter van Ruitenbeek, Annemiek Vermeeren, Willem J. Riedel

12.00 – 13.00 Oral session VIII (**O30-O34**): **COST ACTION Histamine Medicinal Chemistry I**

Chairpersons: Rob Leurs & Rushdie Abuhamdah

12.00-12.15 O30 Pharmacological Characterization of Oxime Agonists of the Histamine H₄ Receptor

Fuqu Yu, Ronald L. Wolin, Jianmei Wei, Pragnya J. Desai, Patricia M. McGovern, Paul J. Dunford, Lars Karlsson and Robin L. Thurmond

12.15-12.30 O31 2-Amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine Derivatives as Ligands of Histamine H₄ Receptor.

Tadeusz Karcz¹, Jadwiga Handzlik¹, Dorota Łażewska¹, Tim Kottke², Roland Seifert³, Holger Stark², Katarzyna Kieć-Kononowicz.¹

12.30-12.45 O32 Discovery and SAR of Pyrimidine Derived Histamine H₄ Receptor Antagonists



Brad M. Savall

12.45-13.00 O33 Is it possible to increase hit rates in virtual screening by multiple focusing? Indexing chemicals for their H₄ receptor antagonism

Salvatore Guccione¹ Danilo Milardi², Matteo Pappalardo³ and Anwar Rayan⁴

13.00-13.15 O34 Fragment optimization leading to H₄ receptor ligands with differential pharmacology

Rob Leurs¹, Maristella Adami², Rogier Smits³, Herman Lim³, Obbe Zuiderveld¹, Iwan de Esch¹ and Gabriella Coruzzi²

13.15-14.00 Lunch

14.00-15.15 Oral session IX (O35-O39) (Sir James Black Session) – Robin Ganellin
introduction: **Histamine Medicinal Chemistry II**

Chairpersons: Robin Ganellin Kerstin Sander

14.00-14.15 O35 Acidic Elements in Histamine H₃ Receptor Ligands

Kerstin Sander^a Yvonne von Coburg^a, Jean-Claude Camelin^b, Xavier Ligneau^b, Oliver Rau^a, Manfred Schubert-Zsilavecz^a, Jean-Charles Schwartz^b and Holger Stark^a

14.15-14.30 O36 *para-t*-Pentylphenoxyalkyl Piperidine Derivatives as Potent Histamine H₃ Receptor Ligands

Kamil J. Kuder¹, Dorota Łażewska¹, Holger Stark², Xavier Ligneau³, Jean-Claude Camelin³, Katarzyna Kieć-Kononowicz¹

14.30-14.45 O37 Systems Biology on histamine H₄ receptor activity

Aurelio Moya-García¹, Carlos Rodríguez², Ian Morilla², Almudena Pino-Ángeles¹, Ignacio Fajardo², Juan Antonio García-Ranea², Francisca Sánchez-Jiménez^{1,2}.

14.45-15.00 O38 Indexing drugs for their cardio-toxicity

Anwar Mahmoud Rayan

15.00-15.15 O39 Application of the bivalent ligand approach to acylguanidines resulted in highly potent and selective histamine H₂ receptor agonists

Tobias Birnkammer, Anja Kraus, Hendrik Preuss, Günther Bernhardt, Stefan Dove, Sigurd Elz, Roland Seifert, Armin Buschauer.



XXXIXth Annual Meeting, 13th-16th July 2010, Durham, England

15.15-15.30 Evotech Abstract

15.45-17.30 **General assembly EHRS** (Debating Chamber, Palace Green)

17.30-19.00 **COST MC Meeting** (Birley Room)

19.30 Farewell Dinner & Prizes/Entertainment "Minus Zero" **Hatfield College Refectory**



Social programme

Tues 13th July

18.00-19.15 Public Lecture

Professor J-C Schwartz (Calman Building, Durham University Science Park)

*“Histamine as a neurotransmitter in brain: from discovery to novel psychotropic drugs-
A short history dedicated to the memory of Sir James Black (1924-2010)”*

19.30 onwards Welcome Reception with buffet & Drinks - Hatfield College Bar

Wednesday 14th July

20.00- onwards Banquet (Lumley Castle)

Friday 23rd April

14.00 onwards Excursion (Beamish) followed by Dinner with Northumbria Pipes - Hatfield College

Saturday 24th April

19.30 Farewell Dinner & Prizes/Entertainment - Rock with “Minus Zero” - Hatfield College

Accompanying Partners Programme

A special Programme organised (Cities of Durham and York) for accompanying Partners will be provided.



Oral Session chairpersons

Session	Chairpersons	
OI COST ACTION Histamine H ₄ receptors	Elena Rivera	Maria Gschwandtner
OII COST ACTION Histamine H ₄ receptors	Ralf Gutzmer	Saskia Nijmeijer
OIII El-Sayed Assem General Histamine topics	Bernie Gibbs	Linda Kay
OIV Histamine in the Brain I	Pertti Panula	Natasha Lethbridge
OV H ₃ receptors in the periphery	Gabriella Coruzzi	Konstantinos Kyriakidis
OVII EHRS Young Investigator Award Symposium	Holger Stark, Beatrice Passani, Ekaterini Tiligada	
OVII Histamine in the Brain II	Patrizio Blandina	Peter van Ruitenbeek
OVIII COST ACTION Histamine Medicinal Chemistry I	Rob Leurs	Rushdie Abuhamdah
OIX Sir James Black Histamine Medicinal Chemistry II	Robin Ganellin	Kerstin Sander

Poster session Chairpersons

P1-7 Katherine Tiligada (Chair) and Konstantinos Papamichael

P8-14 Jian-Sheng Lin and Christelle Anaclet

P15-21 Emanuela Masini and Leonardo Munari

P22-27 Peter Peachell and Tünde Simon



Plenary Sessions

COST Public Lecture: Professor Jean-Charles Schwartz

Histamine as a neurotransmitter in brain: from discovery to novel psychotropic drugs-
A short history dedicated to the memory of Sir James Black (1924-2010)

GB West Lecture: Professor Jean-Charles Schwartz

Clinical applications of pitolisant (BF2.649), an inverse agonist at the H₃ receptor

Professor Patrizio Blandina

Functional implications of Histaminergic Neurons Heterogeneity

Dr Marlon Cowart

Histamine H₃ antagonist properties and efficacy in CNS models

Dr Jorge D. Brioni

Discovery of Novel Agents for the Treatment of CNS Disorders

Dr Nicholas I. Carruthers

Translational approaches towards the identification of a histamine H₃ receptor antagonist
and its' clinical evaluation for the symptomatic treatment of allergic rhinitis

Dr Steve Liu

Histamine H₃ and H₄ receptors – therapeutic opportunities in airway and inflammatory
diseases



Oral sessions (O1-O39) Debating Chamber, Palace Green

O1 Protein Fragment Complementation, A New Way To Look At Human H₄ Receptor Oligomerization

Saskia Nijmeijer¹, Henry F. Vischer¹, Paul L Chazot², Martine J. Smit¹ and Rob Leurs¹

¹*Vrije Universiteit Amsterdam, Leiden/Amsterdam Center for Drug Research, De Boelelaan 1083, 1081 HV Amsterdam, the Netherlands*

²*Centre for Integrative Neuroscience, School of Biological & Biomedical Sciences, Durham University, South Road, Durham DH1 3LE, England*

O2 H₄R ligands induce STAT downstream phosphoregulation

Cathleen Krieg, Violetta Hames, Jutta Haefner, Susanne Diel, Hannelore Borck, Friedhelm Diel

IUG and FB:Oe, Hochschule Fulda, Petersgasse 27, 36037 Fulda, Germany

O3 Role of histamine H₄ receptor in murine dendritic cell functions

Tünde Simon, Valéria László, András Falus

Department of Genetics, Cell- and Immunobiology, Semmelweis University Budapest 1089. Hungary

O4 Human memory Th17 cells express a functional H₄ receptor.

Susanne Mommert, Brigitta Köther, Maria Gschwandtner, Ralf Gutzmer, Thomas Werfel.

Hannover Medical School, Department of Dermatology and Allergy, Division of Immunodermatology and Allergy Research, Ricklinger Str. 5, D-30229 Hannover, Germany

O5 JNJ7777120 Compound: a Potential Candidate for Use as Radioprotector

Vanina A Medina(1,2), Diego Martinel Lamas(1), Maximo Croci(3), Juan P Prestifilippo(4), Eliana Carabajal(1), Juan C Elverdin(4), Rosa M Bergoc(1,2), Elena S Rivera(1)

(1) Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113, ARGENTINA. (2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), ARGENTINA. (3) Instituto de Inmunooncología, Av. Córdoba 3200, Buenos Aires, 1187, ARGENTINA. (4) Cátedra de Fisiología, Facultad de Odontología, Universidad de Buenos Aires, 1113, ARGENTINA.



O6 Impact of H₃/H₄ antagonists on IgE and IgE-regulatory cytokine's synthesis

Roman Khanferyan, Nadegda Milchenko, Yulia Dorofeeva

Kuban State Medical University, Krasnodar, 350063, Russia

O7 Histamine H₄ Receptor activation on human 6-sulfo LacNAc-expressing dendritic cells downregulates their proinflammatory capacity

Maria Gschwandtner¹, Knut Schäkel², Thomas Werfel¹, Ralf Gutzmer¹.

¹ *Hannover Medical School, Department of Dermatology and Allergy, Division of Immunodermatology and Allergy Research, Hannover, Germany*

² *Department of Dermatology, University Hospital Heidelberg, Heidelberg, Germany*

O8 Anti-inflammatory effects of a selective histamine H₄R antagonist in a rat model of carrageenan-induced pleurisy.

E. Masini, R. Mastroianni, C. Lanzi, M.R. Quaranta, ¹Thurmond R.L., ²Pini A., ²Bani D.

Departments of Preclinical and Clinical Pharmacology, ²Anatomy, Histology and Forensic Medicine, Sect. Histology, University of Florence, 50139 Florence, Italy and ¹Johnson & Johnson Pharmaceutical Research & Development; L.L.C., San Diego, CA, USA

O9 Histamine downregulates IL-27, another member of the IL-12 family, via H₂R and H₄R on human monocytes.

Ralf Gutzmer, Brigitta Köther, Maria Gschwandtner, Susanne Mommert, Thomas Werfel.

Hannover Medical School, Department of Dermatology and Allergy, Division of Immunodermatology and Allergy Research, Ricklinger Str. 5, D-30229 Hannover, Germany

O10 Differential Modulation of Human Basophil Function by Ambroxol and Related Secretolytic Analogues.

Bernhard F. Gibbs

Medway School of Pharmacy, University of Kent, Chatham Maritime, Kent, ME4 4TB, UK.



O11 Heterogeneity in the responses of human lung mast cells to Stem Cell Factor

Jessica Wan, Linda J Kay, Suzanne Havard, Peter T Peachell

Academic Unit of Respiratory Medicine, University of Sheffield, The Medical School (Floor M), Beech Hill Road, Sheffield S10 2RX, UK

O12 Histamine Signaling And Metabolism In Solid Organ Transplantation

Hubert G. Schwelberger

Molecular Biology Laboratory, Dep. Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Austria

O13 Increased Pyroptosis in Human Amniotic Epithelial Cells (HAEC) in Chorioamnionitis (CHA) May be Related to Histamine Acting via its H₂ Receptor.

Dariusz Szukiewicz^{1,2}, Witold Rongies³, Danuta Maslinska^{1,4}, Anna Bilska¹, Katarzyna Sawicka¹, Slawomir Maslinski¹

¹*Dept. of General & Experimental Pathology, ²First Dept. of Obstetrics & Gynecology, ³Dept. of Rehabilitation, Second Faculty of Medicine, Medical University School, ul. Zwirki i Wigury 61, 02-091 Warsaw, Poland, ⁴Institute of Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.*

O14 Evidence for a role of histamine in motivation-driving wakefulness, study using knock-out mouse models.

R.X. Guo¹, M. Zhang¹, C. Anaclet¹, Q.L. Yao¹, C. Buda¹, J.P. Sastre¹, H. Ohtsu², O.A.Sergeeva³, H.L. Haas³
and J.S. Lin¹

¹*INSERM-U628, Integrative Physiology of Brain Arousal Systems, Department of Experimental Medicine, Faculty of Medicine, Claude Bernard University, 69373 Lyon, France;* ²*Department of Cellular Pharmacology, Tohoku University, School of Medicine, Sendai 980-8575, Japan;* ³ *Department of Neurophysiology, Heinrich-Heine-University, 40225 Duesseldorf, Germany*

O15 The Histaminergic System Regulates Wakefulness Through a Hypothalamo-telencephalic System and Orexin Cells in Zebrafish

Maria Sundvik¹, Hisaaki Kudo¹, Stanislav Rozov¹, Yu-Chia Chen¹, Walter Schunack² and Pertti Panula¹.

¹*Neuroscience Center and Institute of Biomedicine/Anatomy, POB 63 (Haartmaninkatu 8), 00014 University of Helsinki, Finland*

² *Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, 14195 Berlin, Germany*



O16 The Histaminergic system in Parkinson's Disease

Ling Shan^{1,2}, Chun-Qing Liu¹, Koen Bossers², Rawien Balesar², Joop J. Van Heerikhuize², Juan-Li Wu¹, Natasha Lethbridge³, Paul L. Chazot³, Ai-Min Bao^{1,2} and Dick F. Swaab²

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O17 H₃ receptor-dependent regulation of conditioned reward by ethanol

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O18 Involvement of the brain histaminergic system in the melanocortin MC4 receptor agonist RO27-3225-induced resuscitating effect in haemorrhage-shocked rats – haemodynamic studies

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O19 Histaminergic stimulation of astrocytic NT-3 synthesis: Mediation via histamine H₁, H₂ and H₃ receptors

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Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

O20 Involvement of histamine in the gastroprotection induced by ghrelin in the conscious rat

Gabriella Coruzzi¹, Rob Leurs², Holger Stark³ and Maristella Adami¹

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O21 Histamine H₃, rather than H₄ receptors, participate in regional blood flow regulation in rat model of ulcerative colitis

Wiesława Agnieszka Fogel¹, Katarzyna. Kiec-Kononowicz², Barbara Skrzydło-Radomska³ and Jerzy Jochem⁴

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³Chair and Clinic of Gastroenterology, Medical University of Lublin, ⁴Department of Basic Medical Sciences, Bytom, Medical University of Silesia, Poland

O22 Effects of the H₃ Receptor Inverse Agonist GSK334429 on the Histamine Levels of Cartilage, Oesophagus and Peripheral Blood Vessels in Rats with Adjuvant Arthritis

Konstantinos Kyriakidis, Evangelia Zampeli, Konstantinos Papamichael, Ekaterini Tiligada

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

O23 Complementary and Synergistic Control of Wakefulness by Orexins and Histamine, Demonstrated Using a Double Knockout Mouse Model.

Christelle Anaclet¹, K Ouk¹, G. Guidon¹, C. Buda¹, J.P. Sastre¹, H. Ohtsu², M. Yanagisawa³, HL Haas⁴, P. Franco¹ and J.S. Lin¹

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O24 Major human histamine H₃ receptor isoforms display pharmacological differences

Natasha L. Lethbridge [1], Andrew D Medhurst [2], Paul L Chazot [1]

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O25 Histamine and Dopamine in Alcohol Addiction: Friends or Enemies?

Saara Nuutinen, Tiia Ojala, Jenni Vanhanen, Pertti Panula

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O26 Oleoylethanolamide and Brain Histamine Interact to Regulate Feeding Behaviour

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²*Department of Cellular Pharmacology, Tohoku University, Sendai 980-8575, Japan*

O27 Effects Of Citalopram On Tail Suspension Test Require The Presence Of Neuronal Histamine

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O28 Dopamine-induced arousal depends on the histaminergic system

Olga A. Sergeeva¹, Helmut L. Haas¹, Evgenij Yanovsky¹, Sha Li^{1,2} and Jian-Sheng Lin²

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O29 Central H₁-receptor blockade increases sedation, but does not affect memory in healthy humans

Peter van Ruitenbeek, Annemiek Vermeeren, Willem J. Riedel

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O30 Pharmacological Characterization of Oxime Agonists of the Histamine H₄ Receptor

Fuqu Yu, Ronald L. Wolin, Jianmei Wei, Pragnya J. Desai, Patricia M. McGovern, Paul J. Dunford, Lars Karlsson
and Robin L. Thurmond

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U.S.A.*



O31 2-Amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine Derivatives as Ligands of Histamine H₄ Receptor.

Tadeusz Karcz¹, Jadwiga Handzlik¹, Dorota Łażewska¹, Tim Kottke², Roland Seifert³, Holger Stark², Katarzyna Kieć-Kononowicz.¹

¹ Department of Technology and Biotechnology of Drugs, Jagiellonian University, Medical College, Kraków, 30-688, Poland, ² Institut für Pharmazeutische Chemie, Biozentrum, ZAFES/LiFF/CMP, Johann Wolfgang Goethe-Universität, Frankfurt/Main, 60438, Germany, ³ Department of Pharmacology, Medical School of Hannover, Hannover, 30625, Germany.

O32 Discovery and SAR of Pyrimidine Derived Histamine H₄ Receptor Antagonists

Brad M. Savall

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O33 Is it possible to increase hit rates in virtual screening by multiple focusing? Indexing chemicals for their H₄ receptor antagonism

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O34 Fragment optimization leading to H₄ receptor ligands with differential pharmacology

Rob Leurs¹, Maristella Adami², Rogier Smits³, Herman Lim³, Obbe Zuiderveld¹, Iwan de Esch¹ and Gabriella Coruzzi²

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O35 Acidic elements in histamine H₃ receptor ligands

Kerstin Sander^a Yvonne von Coburg,^a Jean-Claude Camelin,^b Xavier Ligneau,^b Oliver Rau,^a Manfred Schubert-Zsilavecz,^a Jean-Charles Schwartz,^b and Holger Stark^a

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O36 *para*-t-Pentylphenoxyalkyl Piperidine Derivatives as Potent Histamine H₃ Receptor Ligands

Kamil J. Kuder¹, Dorota Łażewska¹, Holger Stark², Xavier Ligneau³, Jean-Claude Camelin³, Katarzyna Kieć-Kononowicz¹

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O37 Systems Biology on histamine H₄ receptor activity

Aurelio Moya-García¹, Carlos Rodríguez², Ian Morilla², Almudena Pino-Ángeles¹, Ignacio Fajardo², Juan Antonio García-Ranea², Francisca Sánchez-Jiménez^{1,2}.

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O38 Indexing drugs for their cardio-toxicity

Anwar Mahmoud Rayan

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O39 Application of the bivalent ligand approach to acylguanidines resulted in highly potent and selective histamine H₂ receptor agonists

Tobias Birnkammer, Anja Kraus, Hendrik Preuss, Günther Bernhardt, Stefan Dove, Sigurd Elz, Roland Seifert, Armin Buschauer.

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Poster sessions (P1-P27) Hatfield College

Poster session I (16.00 – 16.45) Chairpersons: *Ekaterini Tiligada and Konstantinos Papamichael*

P1 Histaminergic modulation of striatal function

Tommas J. Ellender, Icnelia Huerta-Ocampo, Marco Capogna & J. Paul Bolam

MRC Anatomical Neuropharmacology Unit, Dept. Pharmacology, Mansfield Road, OX1 3TH, Oxford, United Kingdom

P2 Pharmacological properties and precognitive effects of ABT-288, a potent and selective histamine H₃ receptor antagonist

Marlon Cowart, Timothy A. Esbenshade, Kaitlin E. Browman, Thomas R. Miller, John L. Baranowski, Kathleen M. Krueger, Victoria Komater-Roderwald, Min Zhang, Gerard B. Fox, Lynne Rueter, Holly M. Robb, Richard J. Radek, Karla U. Drescher, Thomas A. Fey, R. Scott Bitner, Kennan Marsh, James S. Polakowski, Chen Zhao, Arthur A. Hancock, James P. Sullivan, and Jorge D. Brioni.

Abbott Laboratories, Department of Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Park, Illinois 60064-6123. USA

P3 The Effects of Bilastine 20 mg and 40 mg, and Hydroxyzine 50 mg on Actual Driving Performance

Silke Conen, Eef L. Theunissen, Jan G. Ramaekers

Maastricht University, Faculty of Psychology and Neuroscience, Department of Neuropsychology and Psychopharmacology, 6200 MD Maastricht, The Netherlands

P4 Histamine H₂ receptor immunoreactivity in the mouse brain

R.E. Kaisler¹, K. Karlstedt¹, A. Vila², D.W. Marshak², P. Panula¹

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2 University Texas Medical School, Houston, 77030 Texas, US*

P5 The Effects of Properties of Food on Amygdalar Histamine Release in Rats.

Tomoko Ishizuka¹, Noritaka Sako², Tomotaka Murotani³, Atsushi Yamatodani³, Kiyoshi Ohura¹

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P6 Species-directed immunological probes for the H₄ histamine receptor: evidence for multiple roles for the H₄R

Mwape Katebe, Natasha lethbridge, Fiona C, Shenton and Paul L Chazot

Integrative Neuroscience, School of Biological & Biomedical Sciences, Durham University, UK.

P7 Immunological probes for human H₃ histamine receptor isoforms

Natasha Lethbridge, Fiona C, Shenton, Victoria Hann and Paul L, Chazot

Integrative Neuroscience, School of Biological & Biomedical Sciences Durham University, UK.

Poster session II (16.45 – 17.30) Chairpersons *Jian Sheng Lin and Christelle Anaclet*

P8 Identification of H₃ and H₄ Histamine Receptors on Normal and Cystic Fibrosis Epithelial Cells

Jennifer B Stott¹, PL Chazot², N Lethbridge², A Zholos¹, M Ennis¹

1. *Queens University Belfast, Belfast, BT12 6BN, Northern Ireland*

2. *Durham University, Durham, UK*

P9 Histamine H₄ receptor mediated suppression of IL-12 family cytokines

Hannah Bunk, Kristine Roßbach, Manfred Kietzmann and Wolfgang Bäumer

Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Hannover, Germany

P10 Role of H₄ Receptor in Histamine-induced Inhibition of Human Breast Cancer Cell Proliferation

Diego Martinel Lamas(1), Pablo G Brenzoni(1), Noelia Massari(1), Mariel A Nuñez(1), Rosa M Bergoc(1,2), Elena S Rivera(1), Vanina A Medina(1,2)

(1) *Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113, ARGENTINA.* (2) *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), ARGENTINA.*



P11 Histamine H₄ Receptor in Human Melanoma Cells and Tissues.

Noelia Massari(1), Vanina A Medina(1,2), Diego Martinel Lamas (1), Maximo Croci(3), Graciela P Cricco(1), Lorena Sambuco(3), Rosa M Bergoc(1,2), Elena S Rivera(1)

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P12 Histamine Regulates MDA MB 231 Breast Cancer Cell Line Invasive Potential.

Graciela P Cricco, María S Sáez, Nora A Mohamad, Eduardo Valli, Rosa M Bergoc, Elena S Rivera, Gabriela A Martín.

Laboratorio de Radioisótopos. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junín 956 C1113AAB, Buenos Aires, Argentina.

P13 Effects of histamine H₄ receptor ligands on rodent models of acute inflammation

Maristella Adami¹, Cristina Pozzoli¹, Rogier Smits², Iwan JP de Esch², Rob Leurs² and Gabriella Coruzzi¹

¹Department of Human Anatomy, Pharmacology and Forensic Medicine, 43100 Parma, Italy; ²Leiden/Amsterdam Centre for Drug Research, 1081 HV, Amsterdam, The Netherlands

P14 Expression Patterns Of Histamine Receptors In The Gαi2-Deficient Mouse Model Of Colitis.

Ashok K Kumawat¹, Yu-Yuan Götlind^{2,3}, Maria F Fredin⁴, Roger Willén⁵ Hilja Strid¹, and Elisabeth H Hörnquist^{1,3}.

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Poster session III (17.30 – 18.15) Chairpersons: Emanuela Masini and Leonardo Munari

P15 The immunogenic role of microvesicles in asthmatic and healthy pregnant women

Erna Pap, Éva Pállinger, András Falus

Department of Genetics, Cell – and Immunobiology, Semmelweis University, Budapest, 1089 - Hungary



P16 Histamine chloramine modifies adjuvant arthritis in rats

E.Wojtecka-Lukasik¹, P. Rzdokiewicz¹, D.Maslinska², D. Szukiewicz³, W. Schunack⁴ and S. Maslinski^{1,3}

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³*Department of Pathophysiology, Medical University of Warsaw, Poland.*

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P17 Histamine H₂ Receptor Expression in the Human Amnion Epithelium (HAE), Histamine-Evoked Interleukin(IL)-18 Secretion and Intensity of Pyroptosis in Chorioamnionitis (CHA).

Dariusz Szukiewicz^{1,2}, Grzegorz Szewczyk¹, Dariusz Białoszewski³, Danuta Maslinska^{1,4}, Michał Pyzlak¹, Sławomir Maslinski¹

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⁴*Institute of Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.*

P18 Phenotype and distribution of mast cells in cystic meningiomas

Maslinska D¹, Laure-Kamionowska M¹, Szukiewicz D², Wojtecka-Lukasik E³

¹*Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, ²Department of Pathophysiology, Medical University of Warsaw, ³Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland*

P19 Paradoxical effects of the EP₂ agonist, Butaprost, on histamine release from human mast cells

Linda J Kay, Peter T Peachell

Academic Unit of Respiratory Medicine, University of Sheffield, University of Sheffield, The Medical School (Floor M), Beech Hill Road, Sheffield S10 2RX, UK

P20 Control of Human Basophil Activation by the SH2-Containing Inositol 5-phosphatase (SHIP)-1 is Dependent on the Nature of High-Affinity IgE Receptor Engagement.

Claire L. Streatfield and Bernhard F. Gibbs

Medway School of Pharmacy, University of Kent, Chatham Maritime, UK.



P21 Isn't Histamine a unique amine?

Anna Stasiak¹, Mercedes Unzeta², Abdelouahid Samadi³, Jose L. Marco³, W. Agnieszka Fogel¹

¹Department of Hormone Biochemistry Medical University of Lodz, Poland, ²Department of Biochemistry, Autonomus University Barcelona, Spain, ³Institute of Organic Chemistry CSIC, Madrid, Spain

Poster session IV (18.15 – 18.45) Chairpersons: Peter Peachell and Tünde Simon

P22 Comparison of the differentiation capacity of histamine free (HDC KO) and wild type cardiac stem cells

S. Tóth¹, A. Földes², A. Falus¹,

¹Dept. Genetics, Cell and Immunobiology, Semmelweis University, Budapest, 1089 Hungary, ²Dept. Oral Biology, Semmelweis University, Budapest, 1089 Hungary,

P23 Pharmacological Properties of UR-63325, a H₄R Antagonist in Clinical Development

J. Alfón, S. Sánchez-Gómez, A. Fernández, B. Gil, N. Ardanaz, J. Román, A. G. Gómez-Valadés, C. Mascaró, Ll. Gómez, D. Balsa, X. Bartrolí, M. Merlos

Palau Pharma S.A., Avda. Camí Reial 51-57, Palau-solità i Plegamans, 08184 Barcelona, Spain

P24 Synthesis and Structure-Activity Relationships of Conformationally Constrained Cyanoguanidines: Potent and Selective Histamine H₄ Receptor Agonists

Geyer, R., Igel, P., Bernhardt, G., Buschauer, A.

Institute of Pharmacy, Department of Medicinal Chemistry II, University of Regensburg,

D-93040 Regensburg, Germany

P25 An *In Silico* Study of Interactions Between Mammalian Histidine Decarboxylase and its Inhibitor Epigallocatechin 3-Gallate

M. Victoria Ruiz-Pérez, Almudena Pino-Ángeles, Aurelio A. Moya-García, Francisca Sánchez-Jiménez, Miguel A. Medina

Department of Molecular Biology and Biochemistry, Faculty of Sciences, University of Málaga, and CIBER de Enfermedades Raras (CIBERER), E-29071 Málaga, Spain



P26 Dual blockers of histamine H₃ receptors and norepinephrine transporter for the treatment of pain

Tiffany Runyan Garrison, Robert Altenbach, Huaqing Liu, Marina Strakhiva, Arlene Manalli, Tracy Carr, Brian Wakefield, Chen Zhao, Larry Black, Madhavi Pai, Erica Wensink, Anita Salyers, Tom Shaughnessy, Marlon Cowart, Tim Esbenshade, Gin Hsieh and Jorge Brioni

Neuroscience Research, Global Pharmaceutical Research & Development, Abbott Laboratories, Abbott Park IL, 60064

P27 Expression profiling of chemotactic receptors in inflammatory diseases

Petra de Kruijf¹, Pim Koelink², Bin Zheng², Gert Folkerts², R. Leurs¹, P.L. Chazot³, N.L. Lethbridge³, Aletta D. Kraneveld², Martine J. Smit¹

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² *Utrecht Institute for Pharmaceutical Sciences, Department of Pharmacology and Pathophysiology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*

³ *School of Biological and Biomedical Sciences, Durham University, South Road, Durham, United Kingdom*



Plenary Lecture Abstracts



COST Public Lecture:

Histamine as a neurotransmitter in brain: from discovery to novel psychotropic drugs- A short history dedicated to the memory of Sir James Black (1924-2010)

Jean-Charles Schwartz

Bioprojet, France

The progressive disclosing of a histaminergic neuronal pathway in brain and its functions started at the beginning of the seventies first led to the development of non-sedative antihistamines, then, more recently of the H₃-receptor antagonists/inverse agonists which are finding important clinical applications in Neurology and Psychiatry.

The example of Sir James Black's work has been a model for drug development based upon a solid pharmacology

GB West Lecture:

Clinical applications of pitolisant (BF2.649), an inverse agonist at the H₃ receptor

Jean-Charles Schwartz

Pitolisant (BF2.649) is the first H₃ receptor inverse agonist to enter latter stages of clinical development. Clinical trials still ongoing with this compound already indicate that this novel class of drug will find useful applications both as wakefulness and cognitive enhancers.



Translational approaches towards the identification of a histamine H₃ receptor antagonist and its' clinical evaluation for the symptomatic treatment of allergic rhinitis

Nicholas I. Carruthers

Johnson & Johnson Pharmaceutical Research & Development L.L.C., 3210 Merryfield Row, San Diego, CA 92121, U.S.A.

Histamine H₃ receptors are inhibitory auto/hetero-receptors expressed both in the CNS and the periphery and play a role in the regulation and release of several neurotransmitters. Receptor activation by histamine results in the inhibition of neurotransmitter release. Allergic rhinitis is associated with the release of histamine from several cell types, most notably mast cells and, although many of the symptoms of allergic rhinitis can be treated by histamine H₁ antagonists, nasal congestion persists. In the nasal mucosa activation of H₃ receptors by histamine results in a reduction of norepinephrine outflow which contributes to nasal congestion. This rationale has prompted the preclinical evaluation of histamine H₃ antagonists to reverse the effects of nasal blockage elicited by histamine release.

The characterization of JNJ-39220675, a potent and selective histamine H₃ antagonist, in several preclinical models with the emphasis on the correlation of receptor occupancy with efficacy will be presented together with its' clinical evaluation in seasonal allergic rhinitis.



Functional implications of Histaminergic Neuron Heterogeneity

Patrizio Blandina

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Early studies described histamine neurons as a homogeneous cell group in the hypothalamic tuberomammillary nuclei (TMN) projecting to the whole brain (1), but recent reports indicate that they are an heterogeneous cell population. In these studies dual-probe microdialysis was used to block pharmacologically the H₃ receptors (H₃-R) or the GABA_A-R in the TMN, and monitor modifications of histamine release in histaminergic projection areas. Intra-hypothalamic perfusion of H₃ antagonists or bicuculline increased histamine release from the TMN and cortex, but not from the striatum. H₃ antagonists, but not bicuculline increased histamine release from the nucleus basalis magnocellularis (NBM), whereas bicuculline but not H₃ antagonists increased release from the nucleus accumbens. Intra-hypothalamic perfusion with thioperamide increased the time spent in wakefulness. To explore the local effects of H₃-R blockade in the histaminergic projection areas, each rat was implanted with a single probe to simultaneously administer H₃ antagonists and monitor local changes in histamine release. H₃ antagonists increased histamine release from the NBM and cortex significantly, but not from the nucleus accumbens or striatum. H₃-Rs distribution on histaminergic neurons was assessed using double-immunofluorescence with anti-histidine decarboxylase antibodies to identify histaminergic cells and anti-H₃-R antibodies. Confocal analysis revealed an uneven distribution of H₃-Rs on histaminergic somata. In conclusion, the present data suggest that histaminergic neurons establish functionally distinct pathways according to their terminal projections, that are sensitive to selective pharmacological manipulations, and related to independent functions. This arrangement may influence individually defined behaviours resulting from differential histamine release in various brain regions.

(1) Haas H, Sergeeva O, Selbach O *Physiol Rev* 2008;88:1183-1241.



Histamine H₃ antagonist properties and efficacy in CNS models

Marlon Cowart

Senior Chemistry Group Leader, Dept. Neuroscience Research, Abbott Laboratories

Histamine H₃ receptors localized on presynaptic nerve terminals in the CNS modulate the release of key neurotransmitters involved in complex behaviors, and antagonists are being targets as potential treatments for AD, schizophrenia, and pain. Ultimately, beyond the goal of good in vitro potency, agents are also designed to promote efficient target access by having good CNS-penetration and pharmacokinetic properties, and at the same time take into account structural features that minimize undesired properties such as hERG blockade, phospholipidosis, and CYP inhibition. Efficacy in disease models requires that compounds bind to and act at the targeted CNS receptor population; this can be assessed in behavioral models and rationalized by receptor occupancy. Such principles will be discussed, along with drug-likeness profiles.



Discovery of novel agents for the treatment of CNS disorders

Jorge D. Brioni

Associate Director, Neuroscience Research, Abbott Laboratories

The discovery of novel medicines for the treatment of CNS disorders has maintained a constant rate in the last 20 years despite the significant technological advances experienced in the medical area. The increase in the cost of drug discovery and the demand for improved drug efficacy and safety are some of the main drivers at the present time. New medicines are needed for the treatment of Alzheimer's disease, schizophrenia, ADHD and pain, among other areas in neuroscience.

The scientific approach that the industry is taking to accelerate the development of new drugs will be reviewed in this lecture. The preclinical data on the discovery of novel histamine H₃ receptor antagonists for the treatment of Alzheimer's disease and cognitive deficits of schizophrenia will be discussed as these novel agents are presently advancing to Phase 2.



Histamine H₃ and H₄ receptors – therapeutic opportunities in airway and inflammatory diseases

W.L. Steve Liu

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Since its discovery in 1910 by Sir Henry Dale, the biogenic amine histamine has been one of the most widely researched molecules in science. For the pharmaceutical industry, receptors for histamine have proven to be fruitful targets in the discovery of blockbuster drug classes. H₁ receptor antagonists or antihistamines have been in use for more than 50 years in the treatment of allergic disorders, whilst H₂ receptor antagonists have revolutionised the management of gastric ulcer diseases. Although no H₃ receptor antagonists have reached the market, many compounds are in clinical development, notably for the treatment of various CNS conditions. H₄, the most recently discovered histamine receptor subtype is linked to a variety of immune and inflammatory disorders, and selective antagonists are reported to be entering the clinic. This presentation will discuss the therapeutic opportunities offered through targeting of the H₃ and H₄ receptors in treating airway and inflammatory diseases.



Oral sessions Abstracts



O1

Protein Fragment Complementation, A New Way To Look At Human H₄ Receptor Oligomerization

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G protein-coupled receptors (GPCRs) are cell surface proteins involved in cellular communication. Recent research revealed that GPCRs can form complexes consisting of two GPCRs (dimers). Nowadays, even oligomeric structures are revealed and thought to be of functional importance. Such higher order complexes can be formed between one GPCR subtype (homo-oligomerization) or between different subtypes (hetero-oligomerization).

The human histamine H₄ receptor (hH₄R) is a GPCR that is expressed on the surface of immune cells. Previously, our lab used a variety of biochemical and biophysical techniques to show that the human histamine H₄ receptor (hH₄R) forms homo- as well as hetero-oligomers¹.

In this present study we used a new approach, combining bimolecular luminescence /fluorescence complementation (BiLC/BiFC) with resonance energy transfer, to further examine hH₄R oligomerization. In addition, we specifically visualized the localization of hH₄R oligomers at the cell surface using BiFC microscopy.

¹Van Rijn, RM *et al.* Molecular Pharmacology 2006; 69 (4):1194-206



O2

H₄R ligands induce STAT downstream phosphoregulation

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In recent work it was demonstrated that signal transduction (ST) in Th lymphocytes is related to the signal transducer and activator of transcription (STAT) [1]. There is evidence that the Cyclase-triggering GPCR-type histamine (His) receptors (H₁R – H₄R) influence the intracellular JAK/STAT downstream phosphoregulation [2]. His and selective H₄R ligands revealed different STAT1 and STAT6 responses in sensitized lymphocytes. Furthermore, Th17-specific STAT3 also showed responses in sensitized lymphocytes *ex vivo* [3]. This study aims to demonstrate the particular H₄R-induced responses on the STAT downstream regulation in human lymphocytes *ex vivo*, using selective H₄R ligands like JNJ7777120 (JNJ) and the benzoimidazol derivative, JNJ10191584 (VUF 6002).

Peripheral blood was taken from 6 atopic patients (IgE > 500 IU). Blood samples (IgE < 50 IU) and Jurkat cells were used as controls in 3-day cultures [1]. His, JNJ, VUF 6002 and other ligands were added separately or in combination (1 – 10 μM) 4 hours post-plated. The EMSA technique was used for measurement of DNA binding at STAT1oligo nucleotide (5'-IRD700-CAT GTT ATG CAT ATT CCT GTA AGT GAAAA-3'), STAT3 (5'-IRD700-GAT CCT TCT GGG AAT TCC TAG ATC -3') and STAT6 (5'-IRD700-TAG TCA ACT TCC CAA GAA CAG AATCA-3').

As we could show previously, IL-4 and STAT6 were elevated in atopic blood Th2-lymphocytes [1,3]. His inhibited STAT6, but this effect was not changed by JNJ. Surprisingly, the combination of JNJ and His antagonized the inhibitory responses of His on STAT1α/β and STAT6 phosphorylation, and this effect was more pronounced in the atopic group, considering that latent STAT1 is decreased in atopy. Thioperamide, Clobenpropit and JNJ indicated the crucial role of H₄-receptor in the ST processes of blood lymphocytes. Activated STAT1 gene interaction was suppressed in atopy after addition of His and JNJ. STAT6-specific oligoDNA interaction was dramatically increased in the atopic group using JNJ alone (p < 0.001, student t-test). In this context it was of special interest that IL-17E did not respond to His (0.01 mM) in stimulated cell cultures. However, His affected the Th17-specific STAT3 interaction at the gene level. STAT3-DNA binding was elevated in atopy compared to the non-atopic group.

Based on these results it could be suggested that Th1 suppressed Th17 and His could aggravate this regulatory process. Surprisingly, IL-23 showed down-regulatory potency in the sensitized lymphocytes *ex vivo*. Whether IL-17E is a key cytokine in allergy disease remains to be elucidated in future work.

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O3

Role of histamine H₄ receptor in murine dendritic cell functions

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Dendritic cells (DCs) are the linkage between innate and adaptive immunity. Their behaviour is sophisticatedly regulated by numerous signals and mediators of the environment. One of the mediators is histamine which can act through four different receptors. Beside the well known histamine H₁ and H₂ receptors the recently discovered histamine H₄ receptor (H₄R) is also postulated to play a role in a variety of DC functions.

Previously, we have already examined the presence of H₄R on mouse spleen-derived DCs and tested some H₄R-related functions.

In this study, we have performed our examinations partly on mouse DCs isolated from spleen according to Miltenyi protocol and also on DCs differentiated from bone marrow (1). The changes of H₄R expression during DC differentiation was measured by flow cytometry. Differentiated DCs was used in migration studies which were carried out in a Transwell system. The effect of H₄R ligands on stimulated spleen-derived DCs was examined by the help of real-time PCR.

We measured H₄R protein expression on different days of *in vitro* DC differentiation and we have found that H₄R level decreased with time. Formerly the effect of histamine and 4-methyl-histamine (4MH), a potent H₄R agonist, on splenic DC migration was tested and we experienced that it was unaffected. However, our recent studies revealed that a longer histamine treatment during differentiation can enhance the migration capacity of DCs. Central role of DCs in T-cell polarization is exerted by the cytokine production influenced by different factors like histamine. The effect of H₄R agonist 4MH administration was tested on spleen-derived DC cytokine response induced by *in vitro* LPS stimulation. We experienced that mRNA level of IFN γ and IL-1 β , two important cytokines was elevated due to the effect of H₄R agonist.

These results provide further evidences that H₄R has a significant role in the regulation of dendritic cell functions.

(1) Manfred B. Lutz et al. J. of Immun. Met. 1998; 223:77-92



O4

Human memory Th17 cells express a functional H₄ receptor.

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Histamine is an important inflammatory mediator and modulates T cell activation via its four known receptors. The most attractive histamine receptor 4 (H₄R) is expressed on CD4⁺ T cells and in particular on human CD4⁺ Th2 polarized T cells, where functional effects of the H₄R have been described recently (Gutzmer et al. JACI 2009; 123:619-25.). IL-17 producing T cells (Th17 cells) represent a new defined major CD4⁺ T cell subset, distinct from Th1 and Th2 cells.

Here we investigated the expression and function of the H₄R on human Th17 cells obtained from memory T cells.

First Th17 cell polarization was induced by two cytokine combinations. Purified CD4⁺CD45RO⁺ cells were activated by CD3/CD28 ligation and stimulated with TGF-β+IL-6 or IL-1β+IL-23. The IL-17 secretion capacity was proved by quantitative real time PCR and by intracellular staining for IL-17. The H₄R expression was determined by real time PCR and by flow cytometry. The cytokine release was measured by quantitative real time PCR and by cytokine secretion assay. Effects of H₄R stimulation on induction of transcription factors were assessed by electrophoretic mobility shift assays.

In this study, we could confirm recent reports that IL-1β, together with IL-23, markedly enhances IL-17 production and promote differentiation of CD4⁺CD45RO⁺ T cells into Th17 cells, thus these cells are called Th17 cells in our study and were used for further experiments. The expression of the H₄R could be detected at the mRNA and protein level on Th17 cells. Stimulation with histamine or a H₄R agonist resulted in a significant up-regulation of IL-17 mRNA. Furthermore, we could show a significant induction of IL-17 protein secretion upon stimulation with histamine or 4MH at the single cell level. The H₄R agonist 4-methylhistamine (4MH) induced AP-1 in Th17 cells.

In conclusion, the H₄R is functionally expressed on Th17 cells. In inflammatory diseases, such as atopic dermatitis and psoriasis, histamine may act as an exacerbating factor on skin infiltrating Th17 cells.



O5

JNJ7777120 Compound: a Potential Candidate for Use as Radioprotector

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Radiation side effects are inevitable, even with localized radiotherapy. Based on our previous data on the histamine radioprotective effect on highly radiosensitive tissues, in the present work we aimed to determine whether JNJ7777120 compound is able to protect bone marrow, small intestine and salivary glands against ionizing radiation damage. For that purpose, 48 rats were divided into 4 groups. JNJ7777120 and JNJ7777120-irradiated groups received a daily subcutaneous JNJ7777120 injection (10 mg/kg) starting 24 h before irradiation. Irradiated groups received a single dose of 5 Gy on whole-body using Cesium-137 source and were sacrificed 3 or 30 days after irradiation. We evaluated the number of medullar components, bone marrow trophism, oedema, vascular damage, number of intestinal crypts per circumference, and other histological characteristics. We also determined proliferation and apoptosis markers by immunohistochemistry and metacholine-induced salivary secretion of submandibular gland (SMG). Results indicate that JNJ7777120 treatment reduced the grade of aplasia, and substantially prevented bone marrow replacement by adipose tissue produced by ionizing radiation, preserving medullar components. Furthermore, JNJ7777120 diminished mucosal atrophy, oedema and preserved villi and the number of crypts after radiation exposure (240±8 vs. 165±10 in untreated and irradiated rats). Additionally, JNJ7777120 completely reversed the reduced salivation induced by radiation, significantly conserved glandular mass with normal appearance, preserved structure organization of secretor granules and reduced apoptosis in SMG. We conclude that JNJ7777120 compound prevents radiation-induced damage on bone marrow, small intestine and SMG being of potential clinical value for patients undergoing radiotherapy.

We thank Dr. Nicholas Carruthers from Johnson & Johnson Pharmaceutical Research & Development for the JNJ7777120 compound.



O6

Impact of H₃/H₄ antagonists on IgE and IgE-regulatory cytokine's synthesis

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Previously it have been shown that several imidazole and non-imidazole H₃/H₄ histamine receptor antagonists are involved in IgE synthesis by peripheral blood mononuclear cells (PBMC) from healthy donors as well as allergic subjects (R.Khanferyan et al., 2002-2009). It was suggested that the modulation of IgE synthesis induced by H₃/H₄ antagonists and agonist as well as by histamine may be due to influence on IgE-regulatory cytokine synthesis. In the present study, IL-4, IL-10, IL13 and γ IFN levels were assessed by ELISA (Diaclon), the supernatants of PBMC were assayed for total IgE by ImmunoCAP FEIA method (Phadia). It has been shown that H₃/H₄ histamine receptor antagonist Imoproxifan (IMP) in a concentration-dependent manner increased most notably IL-4 and IL-10 synthesis. IMP increased both healthy donor and allergic subject PBMC IL-10 synthesis, the latter group seeing increased synthesis when assessed during the pollen season but decreased synthesis during remission ($p < 0.05$). IMP decreased an IgE stimulatory effect of histamine. H₃/H₄ blockade had a co-stimulatory effect on IL-10 synthesis induced by high concentrations (10^{-5} M) of histamine in healthy donors and allergic subjects during the pollen season. High concentrations of IMP increased the IFN γ –suppressive effect of histamine, while low concentrations (10^{-8} M) increased IFN γ synthesis. High concentration of IMP augmented the effect of histamine increasing IL-4 levels while low concentrations had no impact. Both concentrations of IMP had only slight or no effect on IL13 synthesis. Thus, H₃/H₄ histamine receptor antagonist modulates an IgE synthesis in healthy donors and allergic subjects mainly via influence on IL4 and IL10 synthesis.

Acknowledgement: The authors would like to thank Prof. W. Schunak providing H₃/H₄ compounds for this investigation



O7

Histamine H₄ Receptor activation on human 6-sulfo LacNAc-expressing dendritic cells downregulates their proinflammatory capacity

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6-sulfo LacNAc dendritic cells (slanDC) are a newly identified DC subpopulation in human blood. They are described as highly proinflammatory cells, due to their outstanding capacity to produce TNF- α and IL-12 and to prime antigen-specific T cell responses. Since effects of histamine on various types of antigen presenting cells have been already shown, we investigated the role of histamine receptors on slanDC, particularly the most recently identified histamine H₄ receptor (H₄R). The expression of histamine receptors was evaluated by real-time PCR and flow cytometry. Cytokine production in response to H₄R stimulation was assessed by intracellular flow cytometric staining and enzyme-linked immunosorbent assay. We show that slanDC express the H₁R, H₂R and H₄R on mRNA and the H₄R on protein level. We could not observe differences in basal H₄R expression in patients with atopic dermatitis and psoriasis as compared to healthy controls, but in atopic dermatitis patients the H₄R was upregulated after stimulation with IFN- γ . When stimulated with LPS in the presence of histamine or H₄R agonist, slanDC produced substantially lower levels of the proinflammatory cytokines TNF- α and IL-12. Pre-incubation with the selective H₄R antagonist JNJ7777120 blocked these effects. In contrast, the production of IL-10 was not affected by H₄R activation on slanDC. Thus, slanDC represent another type of antigen presenting cell that can be influenced by H₄R ligands, and H₄R agonists might have therapeutic potential in downregulating immune reactions.



O8

Anti-inflammatory effects of a selective histamine H₄R antagonist in a rat model of carrageenan-induced pleurisy.

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The histamine H₄ receptor (H₄R) is widely expressed in cells of immune origin, and play an important role in inflammatory processes (Thurmond *et al*, 2008). We have previously demonstrated that a H₄R antagonist decreases the inflammatory response and airway hyperreactivity in a guinea pig model of asthma (Masini, 2009).

The aim of the present investigation is to evaluate the effect of the selective H₄R antagonist JNJ7777120 (JNJ) in a widely used *in vivo* model of acute inflammation in the rat, namely carrageenan-induced pleurisy, whose cellular and molecular mechanisms are well characterized (Ceccarelli *et al*, 2009)

A 60-min pre-treatment with compound JNJ (5-10 mg · Kg⁻¹ b.wt., given intra-pleurally) attenuated the recruitment of leucocytes in the lung tissue and the pleural exudate, inhibited the induction of inducible nitric oxide synthase and cyclooxygenase-2, thereby abating the generation of harmful nitric oxide and pro-inflammatory prostaglandins such as PGE₂ and PGF_{1α}, reduced the inflammation-induced oxidative/nitrosative lung tissue injury, as shown by tissue malondialdehyde, 8-OH-d-guanosine and nitrotyrosine production, and blunted the local generation of cytokines such as IL-1β and TNF-α . All these parameters were markedly increased by intra-pleural administration of λ-carrageenan (1% w/v in 0.2 ml sterile saline) in the absence of any pre-treatment.

This study provides further insight that H₄R antagonism can modulate the inflammatory response and has potential therapeutic utility in the treatment of several inflammatory diseases.

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09

Histamine downregulates IL-27, another member of the IL-12 family, via H₂R and H₄R on human monocytes.

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Recent studies showed that histamine can inhibit IL-12p70 production by human antigen presenting cells via H₂R and H₄R stimulation. We sought to investigate if also the expression of IL-27, another member of the IL-12 family that is involved in initiation of Th1 responses, is modulated by histamine. Thus, we stimulated human monocytes with histamine or histamine receptor specific ligands, induced IL-27 expression by a second stimulus (i.e., the toll like receptor ligands polyI:C or LPS + IFN- γ) and measured IL-27 by real time quantitative PCR and ELISA.

Stimulation with histamine resulted in a significant downregulation of the two IL-27 subunits, p28 and EB13, on the mRNA level. Histamine downregulated IL-27 also on the protein level, however, other cytokines such as IL-6, TNF- α and IL-10 were not downregulated by histamine. The downregulation of IL-27 protein could also be obtained with the H₂R agonist amthamine and the H₄R agonist 4-methylhistamine and blocked with ranitidine and JNJ7777120, respectively. In contrast, agonists of the H₁R and H₃R had no effect. IL-27 can induce the chemokine IP-10 in keratinocytes. Supernatants of monocytes stimulated with LPS + IFN- γ (containing high amounts of IL-27) induced significantly more IP-10 than supernatants from monocytes stimulated with histamine + LPS + IFN- γ (containing low amounts of IL-27). This difference could be abolished by adding IL-27 to the culture supernatants of the histamine samples.

In conclusion, histamine blocks not only the production of IL-12p70 but also of other members of the IL-12 family, such as IL-27. This blockade is mediated via H₂R and H₄R and appears to be fairly selective, since other cytokines were not downregulated. The pathogenesis of Th1-dominated inflammatory diseases might be influenced by this mechanism, in particular if increased concentrations of histamine are present at sites of inflammation, such as chronic eczema and psoriasis.



O10

Differential Modulation of Human Basophil Function by Ambroxol and Related Secretolytic Analogues.

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Ambroxol is a secretolytic agent that was originally developed from vasicine, a natural alkaloid found in *Adhatoda vasica*, which has been employed to treat asthma, bronchitis and rheumatism. We have previously reported that ambroxol inhibits IgE-dependent mediator secretion from human mast cells and basophils, which both play an important role in supporting allergic inflammation. In the present study, the mechanisms involved in the inhibitory properties of ambroxol were assessed, also in comparison to the related analogues vasicine, bromhexine and sputolysin. In comparison to ambroxol, however, which significantly reduced IgE-dependent histamine release from basophils above 10 μ M, sputolysin and vasicine produced only moderate inhibitory effects at 1 mM. In stark contrast, bromhexine displayed marked *in vitro* toxicity, including histamine release, above 100 μ M. The inhibitory actions of ambroxol at concentrations below 1 mM were not toxic and entirely reversible. Ambroxol was shown to reduce IgE-dependent p38 MAPK as well as ERK1&2 phosphorylations in basophils, unlike bromhexine, sputolysin and vasicine. Furthermore, ambroxol attenuated extracellular calcium influx in basophils but preliminary observations show that it has less effect on intracellular calcium mobilization in basophils stimulated with anti-IgE in calcium-free buffer. These results highlight the superior inhibitory properties of ambroxol compared to related secretolytic analogues in terms of blocking basophil degranulation. Additionally, the data suggest that the mode of action of ambroxol lies primarily in preventing signalling that controls extracellular calcium influx into basophils. The therapeutic potential of ambroxol as an anti-allergic agent is further underscored by these results.



O11

Heterogeneity in the responses of human lung mast cells to Stem Cell Factor

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Stem Cell Factor (SCF) promotes mast cell development and survival. Moreover, SCF enhances the responses of human lung mast cells to IgE-directed ligands. However, our recent work suggests that SCF alone may activate mast cells. The principal aim of this study was to explore the effects of SCF on human lung mast cells further.

Mast cells were isolated by physical and enzymatic disruption of human lung tissue and further purified by flotation over Percoll gradients. Mast cells were activated with or without SCF (or anti-IgE) for 30 min for the release of histamine, prostaglandin D₂ (PGD₂) and cysteinyl-leukotrienes (cys-LT). Alternatively, mast cells were activated with stimuli for 8 h and the generation of a number of cytokines (IL-1-beta, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, TNF-alpha and VEGF) was determined by Cytometric Bead Array immunoassay (BD Biosciences).

In agreement with previous studies, SCF (10 ng/ml) caused significant ($P < 0.05$) enhancement of anti-IgE-induced histamine, PGD₂ and cys-LT generation from human lung mast cells ($n = 10-30$). At higher concentrations, SCF (100 ng/ml) alone caused substantial levels of histamine release (20 to 45%) and cys-LT generation (4 to 14 ng cys-LT/ 10^6 mast cells) in about a third of all mast cell preparations ($n = 20$). SCF was even more effective as a stimulator of PGD₂ generation (9 to 95 ng PGD₂/ 10^6 mast cells) since the majority of preparations, even those that hardly generated histamine or cys-LT in response to SCF, produced PGD₂. In studies investigating cytokine generation ($n = 9$), neither anti-IgE (2 μ g/ml) nor SCF (100 ng/ml) induced particularly effective responses but in some preparations substantial synergistic increases in some cytokines (IL-10, IL-13, IL-17) were observed when mast cells were exposed to both stimuli simultaneously.

These studies identify SCF not just as an enhancer but as a direct activator of human lung mast cell responses.



O12

Histamine Signaling And Metabolism In Solid Organ Transplantation

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Early allograft dysfunction, as well as long-term graft loss, still represents major obstacles to successful organ transplantation. Besides immunological reactions due to donor and recipient incompatibility, ischemia/reperfusion injury of the transplanted organ is the major process negatively affecting allograft function early after transplantation and long-term graft survival.

Previously we have been studying ischemia/reperfusion associated damage of grafted organs in mouse and rat models of heart and kidney transplantation and in patients, focusing on new biomarkers for early detection of graft damage and on cytokine and chemokine signaling leading to infiltration of inflammatory cells into the graft. Although the role of histamine in ischemia and reperfusion has been studied in various animal models its specific role in solid organ transplantation has only been poorly explored. Therefore, we would like to apply established procedures but also new techniques emerging from COST Action BM0806 to explore histamine signaling through the different histamine receptors and receptor subtypes and its metabolism in the course of solid organ transplantation.

Besides a huge collection of RNA, cDNA, protein and histochemical samples from numerous syngeneic and allogeneic animal transplantation experiments with short-term and long-term follow-up that are ready to use for expression and protein localization experiments, the well-established models can easily be adapted for investigating treatment with specific histamine receptor agonists and antagonists and for testing of various gene knockout settings. Furthermore, our collection of tissue and body fluid samples from patients undergoing solid organ transplantation facilitates comparison of results obtained in animal studies with the situation in humans.



O13

Increased Pyroptosis in Human Amniotic Epithelial Cells (HAEC) in Chorioamnionitis (CHA) May be Related to Histamine Acting via its H₂ Receptor.

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Pyroptosis, or caspase 1-dependent form of programmed cell death, is inherently inflammatory and associated with antimicrobial responses. Caspase-1 is activated by protein complex termed the pyroptosome, that cooperates with toll-like receptors. Histamine induces toll-like receptor 4 expression on epithelial cells and histamine H₁ and H₂ receptors are present in HAEC. We examined comparatively (CHA vs normal controls) influence of histamine and H₁/H₂ receptor status on lipopolysaccharide (LPS)-induced pyroptosis in HAEC. HAEC were isolated from the amnion obtained after term pregnancies, normal (group II; N=12) and complicated by CHA (group I; N=12), using Okita's method. Altogether 72 cultures were established and cultured for 12 days in normoxia in Ham's F12 and Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. In respective subgroups histamine (100µM) was added with/without H₁ and H₂ antagonists, mepyramine (10µM) and cimetidine (10µM), respectively. Influences of caspase-1 inhibitor and anti-interleukin(IL)-18 antibody were also examined in respective controls. At Day 10, the cultures were exposed to LPS (1 µg/ml) for 48h. At Day 12, the cultures were terminated and quantitative determination of apoptosis (including pyroptosis) was performed using an immunoenzymatic assay with antibodies against a neo-epitope of cytokeratin 18 (an pyroptosis marker). Intensity of apoptosis was significantly (p<0.05) higher in group I. Histamine produced significant increase in intensity of pyroptosis, proportionally higher in group I than in the group II (87.6±14.6 vs 32.8±10.1 %±SEM), whereas H₂ blockade reduced this effect nearly to the levels observed initially (apoptotic activity before administration of histamine). H₁ receptor antagonist did not significantly affect pyroptosis intensity in all studied cultures. In conclusion, overexpression of H₂ in CHA may influence Caspase1-dependent cells death. Further studies on H₂ expression in CHA are needed.

O14

Evidence for a role of histamine in motivation-driving wakefulness, study using knock-out mouse models.

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Histamine (HA) and orexins are both considered as brain wakefulness(W)-promoting systems. In order to study their respective role in promoting W associated with the behavioural context of motivation (i.e., motivation-driving W), histidine-decarboxylase (HDC, HA synthesising enzyme) or orexin knockout (KO) mice and their respective wild type (WT) littermates were chronically implanted for simultaneous EEG and sleep-wake monitoring under baseline conditions (Water and food *ad libitum*, 12 h light/dark cycle with lights on at 7 a.m.) and following a test of motivation. The test consisted of introducing into the mouse barrels some palatable food (a piece of nougat and caramel and a grain of corn), hanged up with a height difficultly reachable by mice. We found that in WT animals, the introduction of the palatable food, either during the lightness or darkness, elicited behavioural activation, manifested as an increased locomotion and numerous attempts to climb, to catch and to consume the food. As a result, these mice remained highly awake during the period where the food was present (4h or more). HDC KO mice showed deficient performance, manifested as less attempts towards the food and a significant decrease in W compared to that of their littermates. Pretreatment with alpha-FMH (specific inhibitor of HDC) prevented the increase in W faced with the palatable food in WT mice but had no effect on the sleep-wake states in HDC KO mice. Finally, when orexin KO mice were subjected to the same test, they showed slightly enhanced performance compared to their WT counterparts in terms of behavioural activation and induced W. Our data indicate that HA, but not orexins, is involved in maintaining motivation-driving W that is indispensable for further behavioural performance. These results also further support our hypothesis according to which although both HA and orexins are involved in promoting W, their respective role is distinct under the different behavioural contexts.



O15

The Histaminergic System Regulates Wakefulness Through a Hypothalamo-telencephalic System and Orexin Cells in Zebrafish

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Histamine and hypocretin/orexin have emerged as alertness/wakefulness promoting systems in the vertebrate brain. Both systems have cell bodies in the hypothalamus and are involved in sleep-wakefulness, and lack of orexin gives rise to narcolepsy. Persons suffering from idiopathic hypersomnia have decreased histamine levels, suggesting that histamine in humans is a crucial regulator of alertness/wakefulness. We were interested to understand how histamine is involved in alertness/wakefulness and if lack of histamine affects the developing orexin system. We studied if transient gene knockdown of histidine decarboxylase (*hdc*) with morpholino-oligonucleotides (MO) in larval zebrafish would affect the response of fish to change in light conditions. Animals were treated with histamine receptor ligands to evaluate if the response was mediated through the receptors. Further, we identified the histamine receptor mRNA profile of dorsal telencephalon by *in situ* hybridization and the fiber projections of histamine neurons by immunohistochemistry. Orexin neuron numbers were detected by ISH and mRNA levels by qPCR. Translation inhibition of *hdc* reduced spontaneous activity during wakefulness, whereas no difference compared to normal larvae was observed during the night. During the light period, the dark-induced startle response was deficient in *hdc* morphants. The changes were partly normalized by a histamine H1 receptor agonist. *hdc* MO reduced histamine levels up to 5% of normal in five-day old fish. The main target of histaminergic fibers was dorsal telencephalon, which also was the only site where both histamine H1 and H3 receptor were strongly expressed. Following *hdc* MO the number of orexin-expressing neurons and orexin mRNA expression were strongly reduced in the hypothalamus. Thus, histamine in zebrafish may have a dual effect on wakefulness: a direct one through histamine receptors in dorsal telencephalon and through the hypothalamic orexin system.



O16

The Histaminergic system in Parkinson's Disease

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Background

The hypothalamic tuberomammillary nucleus (TMN) is the exclusive source of neuronal histamine. There are conflicting results on the alterations in the TMN in Parkinson's disease (PD). It was claimed that the abundant presence of Lewy (LBs) and Lewy neurites (LNs) in the TMN of PD patients would result in a strong degeneration of the TMN. On the other hand, experimental data indicated an activation of the TMN that was presumed to accelerate the degeneration of the substantia nigra (SN) in PD. We aimed to clarify this controversy.

Methods

We studied histamine production by quantitative *in situ* hybridization for the rate-limiting enzyme of histamine production, histidine decarboxylase (HDC), in postmortem human brain from PD patients (6 early, preclinical PD; 9 late PD) and 15 well-matched controls. In addition, we measured, by HPLC, histamine in postmortem cerebrospinal fluid (CSF) of 17PD patients and 19 matched controls. Moreover, we performed qPCR in postmortem SN from 7 PD patients and 8 matched controls to determine changes in the expression of histamine N-methyltransferase (HNMT), the histamine inactivating enzyme, and of histamine receptors H₁R, H₂R, H₃R and H₄R. Furthermore, we performed immunocytochemistry (ICC) of H₃R in the SN from 8 PD and 7 matched controls.

Results

No significant alteration was found in HDC-mRNA expression in the TMN in PD patients, nor in the histamine CSF levels. The amount of LBs and LNs, as stained by anti- α -synuclein was significantly increased in the TMN in late-stage PD ($P < 0.0001$), but not in the early-stage PD. There was no correlation between HDC-mRNA expression and the amount of LBs and LNs

In the SN, HNMT-, H₁R-, H₂R- and H₄R-expression were unaltered in PD, while H₃R-mRNA expression was significantly lower ($P = 0.04$). ICC identified H₃R-containing neurons and fibers in the TMN, and neurons and sporadic fibers in the SN. The H₃R staining was decreased the SN of PD.

Conclusion

Despite the abundant presence of LBs and LNs, HDC mRNA in the TMN and CSF histamine levels remain unaffected in PD. However, in the SN, H₃R mRNA and protein levels are decreased in PD.

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O17

H₃ receptor-dependent regulation of conditioned reward by ethanol

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Previous studies have suggested that histamine and H₃ receptor (H₃R) modulate the rewarding effects of alcohol. Here we examined the role of H₃R in ethanol induced conditioned place preference (CPP) in DBA/2J mice using H₃R antagonist ciproxifan and H₃R agonist immpip. An unbiased CPP paradigm was used with cage floor materials as conditioning cues. The unbiased CPP protocol consisted of habituation, conditioning sessions (four ethanol and four saline pairings) and place preference test. On conditioning sessions each mouse was injected with ethanol or saline on alternating days and put to the test cage for 5 minutes. Ciproxifan (3 mg/kg, i.p.) or immpip (30 mg/kg, i.p.) was administered 30 min prior to ethanol conditioning. The place preference was tested 24 h after the last conditioning session. Immediately after saline injection mice were placed in the center of the test cage so that half of the cage floor was covered with metal and the other half covered with plastic. The time spent during 30 min in different zones of the cage and the total distance moved were registered. Ethanol induced a significant place preference in mice that were conditioned with ethanol alone. In contrast to our previous study done in 129/Sv mice, ciproxifan seemed to inhibit the rewarding effect of ethanol. Immpip did not seem to have a clear effect on ethanol reward. Interestingly, when preference was tested again five days after initial testing, we found no preference in animals treated with ethanol alone whereas immpip had a tendency to prolong the ethanol-induced place preference. This suggests that ethanol reward remains longer in animals pretreated with H₃R agonist. More studies will be needed to verify these findings. Our ongoing study will also reveal whether a selective non-imidazole based H₃R antagonist affects ethanol reward. Overall our results are consistent with the hypothesis that H₃R-ligands have an effect on reward circuit and in ethanol addiction.

O18

Involvement of the brain histaminergic system in the melanocortin MC4 receptor agonist RO27-3225-induced resuscitating effect in haemorrhage-shocked rats – haemodynamic studies

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Studies of recent years revealed the existence of a vagus nerve mediated cholinergic anti-inflammatory pathway, activated after brain melanocortin receptor stimulation [1]. Melanocortin MC4 receptor agonists, acting via this pathway, could have a protective role against multiple organ failure following haemorrhagic shock [2], however, particular central mechanisms involved are still unclear. It is of note that the histaminergic system is implicated in the activation of compensatory mechanisms in the response to stimuli disturbing circulatory homeostasis, including critical hypovolaemia [3]. Therefore, the present study was undertaken to examine a possible involvement of the central histaminergic system in the selective MC4 receptor agonist RO27-3225 (64 µg/kg, iv)-induced resuscitating effect. Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats subjected to severe haemorrhagic hypotension, with mean arterial pressure (MAP) stabilized at 20-25 mmHg. RO27-3225 administered to haemorrhage-shocked rats evoked long-lasting rises in MAP and heart rate (HR), with a subsequent increase in renal, mesenteric and hindquarters blood flows. Haemodynamic effects were completely blocked in rats after bilateral cervical vagotomy, and partially inhibited by a pre-treatment with the inhibitor of L-histidine decarboxylase S(+)-α-fluoromethylhistidine (0.5 mg, icv), as well as the H₁ and H_{3/4} receptor antagonists chlorpheniramine (19.5 µg, icv) and thioperamide (20.4 µg, icv), respectively. In addition, pre-treatment with the inhibitor of histamine N-methyltransferase SKF 91488 (100 µg, icv) evoked an increase in HR, while the H₂ receptor antagonist ranitidine (35 µg, icv) had no effect on RO27-3225-induced resuscitating action. In conclusion, the results of our haemodynamic studies demonstrate for the first time an involvement of the brain histaminergic system in the cholinergic anti-inflammatory pathway activation by a selective MC4 receptor agonist, in a haemorrhagic shock model in rats.

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O19

Histaminergic stimulation of astrocytic NT-3 synthesis: Mediation via histamine H₁, H₂ and H₃ receptors

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Neurotrophin-3 (NT-3) is produced by astrocytes, in addition to neurons, and monoamine neurotransmitters play a role in controlling NT-3 synthesis. The impact of histamine (HA) on the regulation of NT-3 synthesis in astrocytes has not been studied therefore we focused our present study on the active involvement of multiple histaminergic receptor and intracellular mechanisms in the regulation of NT-3 production by HA in cultured rat cortical astrocytes.

HA (1 μ M) significantly and transiently elevated NT-3 mRNA levels by 2.2-fold after 30 min of incubation following by 2.1-fold increase in NT-3 intracellular levels after 6 h. Its stimulation was partly inhibited by selective H₁, H₂ and H₃ antagonists. NT-3 levels in astrocytes were increased by selective H₁, H₂ and H₃ agonists as well as by adenylyl cyclase activation (by forskolin), PKA activation (by dBcAMP), PKC activation (by phorbol 12-myristate 13-acetate (TPA)) and mobilization of intracellular Ca²⁺ (by ionophore A23187). However, none of the tested specific agonists or mediators of the intracellular histaminergic pathways were able to reach the level of HA's stimulatory effect. HA-induced increase in NT-3 cellular levels were significantly reduced by H-89 (PKA inhibitor), staurosporin (PKC inhibitor), KN-62 (CaMK II inhibitor) and desensitizing pretreatment with TPA. MAP kinase cascade inhibitor PD98059 completely blocked the stimulatory action of HA and all selective agonists. Using quantitative RT-PCR we confirmed that cultured rat cortical astrocytes express not only H₁ and H₂ receptors already identified by radioligand binding studies but also H₃ receptors which were sensitive to pertussis toxin.

In conclusion, the synthesis of astrocytic NT-3 stimulated by HA is an adaptable process using several parallel histaminergic pathways: H₁-receptor pathway, mediated via activation of PKC and CaMK II, H₂-receptor pathway mediated via cAMP/PKA and the novel H₃-receptor pathway, coupled to Gi/o protein and mediated via activation of several second messengers. The observed H₁, H₂ and H₃ receptor crosstalk in histaminergic regulation of NT-3 leads to the convergence of these pathways at the level of MAP kinase activity which in the next step triggers the increased expression and subsequently the synthesis of NT-3 in astrocytes.



O20

Involvement of histamine in the gastroprotection induced by ghrelin in the conscious rat

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Ghrelin is a 28 amino acid peptide recently identified in rat and human gastrointestinal (GI) tract, which serves as the endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R) (Kojima et al. 1999). Ghrelin is most abundant in the stomach, and particularly in neuroendocrine cells of the gastric mucosa, i.e. the A cells; besides stimulating appetite and food intake, this peptide may alter GI functions, including acid secretion, mucosal defense and motility (Peeters, 2005). A release of histamine by ghrelin has been also hypothesized (Konturek et al. 2007). In the present study the effects of ghrelin were investigated in conscious rats against the gastric damage induced by concentrated acid, and the involvement of histamine was studied by the use of selective ligands. Ghrelin (20-80 µg/kg, i.p.) induced a significant reduction (about 60%) of the gastric lesions caused by intragastric administration of 0.6 N HCl. The effect was prevented by prior administration of the ghrelin receptor antagonist GHRP-6 (100 µg/kg, i.p.) and by the selective histamine H₃-receptor antagonist, compound UCL2138 (30 mg/kg s.c.). This compound was ineffective per se and significantly reduced the protective effect exerted by the selective H₃ receptor agonist immethridine (30 mg/kg s.c.). These data confirm and enlarge previous studies showing protective effects of ghrelin against ethanol-induced damage; in addition clearly indicate that ghrelin releases histamine in the rat gastric mucosa, which in its turn activates H₃ receptors, leading to enhancement of gastric mucosal defense.

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O21

Histamine H₃, rather than H₄ receptors participate in regional blood flow regulation in rat model of ulcerative colitis

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Rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS)- induced ulcerative colitis showed normalisation of increased regional blood flow and a significant health improvement after being treated with thioperamide, a dual H₃/H₄ receptor blocker (1). In order to identify which of the two components underpinned that effect, two drugs, namely DL-76 and JNJ 7777120, were used that selectively inhibit H₃ or H₄ receptors, respectively.

Experimental ulcerative colitis was induced in ketamine/xylazine (100 mg/kg + 10 mg/kg, ip) anaesthetised Wistar rats by a single intracolonic injection of TNBS (25 mg dissolved in 0.8 ml of 37% ethanol), whilst the control animals received 0.9% saline solution (0.8 ml). The rats were treated daily for 7 days with either DL-76 (6 mg/kg, sc), JNJ 7777120 (10 mg/kg, sc) or saline (0.2 ml, sc).

The colonic haemodynamics measurement was performed after the therapy completion. The rats were anaesthetised and, following a midline laparotomy, the inferior mesenteric artery blood flow (IMBF) was measured by a Transit Time Flowmeter type 700 and the electrode type 1RB (Hugo Sachs Elektronik, Germany). As expected, in the rats with colitis, IMBF was significantly higher than in healthy controls, i.e., 3.55 ± 0.87 vs. 1.86 ± 0.69 ml/min ($p < 0.05$). In the colitic-DL-76 treated rats, IMBF was similar to that in the control ones, being 2.37 ± 0.67 ml/min, whereas in the rats treated with JNJ 7777120, IMBF did not differ from the untreated colitic rats, amounting to 3.43 ± 0.74 ml/min.

The inflammatory indices- macroscopic mucosal damage score, as well as mucosal myeloperoxidase activity were lower in DL-76 treated colitic rats than in the other colitic rats- those untreated, as well as JNJ 7777120 treated, indicating the benefits of anti-H₃ receptor therapy. In conclusion, the results suggest that histamine H₃, rather than H₄ receptors, participate in regional blood flow regulation in rat model of ulcerative colitis. This is compatible with the presence of H₃ receptors in the intestine (2).

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O22

Effects of the H₃ Receptor Inverse Agonist GSK334429 on the Histamine Levels of Cartilage, Oesophagus and Peripheral Blood Vessels in Rats with Adjuvant Arthritis

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Histamine (HI) plays a role in arthritic systemic inflammation by modulating HI receptor-mediated processes [1]. The H₃ receptor (H₃R) is mainly, yet not exclusively, localised in the central nervous system (CNS) and it has been implicated in the modulation of neurotransmission, pain and inflammation. The selective H₃R inverse agonist GSK334429 demonstrated efficacy in models of pain [2], whilst H₃R actions in the periphery remain elusive. This study examined the effects of GSK334429 on the HI content of cartilage, oesophagus and large arteries and veins in a rat model of adjuvant arthritis. Male Wistar rats of 200-250g bw received complete Freund's adjuvant (CFA) i.d. and/or 1-3mg/kg GSK334429 i.p. Following sacrifice at day 20, cartilaginous tissue from ribs #9-10, the oesophagus, abdominal aorta (AA) and inferior vena cava (IVC) were dissected out. Tissue HI was quantified fluorometrically [1]. Differences between treatments and/or tissues were located by appropriate statistical analyses. In normal rats, 1 and 3mg/kg GSK334429 reduced oesophageal and AA HI levels, respectively, while the cartilage HI content was unaffected. CFA administration resulted in the development of arthritic signs in the animal paws and in statistically significant increases of HI content in the cartilage and oesophagus. HI levels decreased in AA but remained unchanged in IVC. In CFA-challenged animals, GSK334429 treatment had no effect on either cartilage or IVC, while it decreased HI levels in the oesophagus and AA. The results provided the first evidence towards H₃R functionality in peripheral blood vessels and oesophagus, but not in the cartilage. The (patho)physiological significance of the differential actions of GSK334429 in peripheral tissues is under investigation.

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O23

Complementary and Synergistic Control of Wakefulness by Orexins and Histamine, Demonstrated Using a Double Knockout Mouse Model.

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We have shown that mice lacking histamine(HA) are characterized by a somnolence, notably a deficit of wakefulness(W) when high vigilance is required; whereas orexin(Ox) knockout(KO) mice are distinguished by a W deficit faced with a motor challenge. We then suggested that HA and Ox exert a distinct, but complementary control of W. To access the synergies of the two waking systems, we have characterized the sleep-wake phenotypes of KO mice lacking HA and Ox.

The double KO mice were obtained by crossing KO mice lacking HA and those lacking orexins. The model was validated by PCR and immunohistochemistry showing the absence of HA-synthesizing and prepro-orexin genes and of HA and Ox neurons. EEG and sleep-wake recordings were performed under baseline conditions and following behavioral or/and pharmacological tests.

Our double KO mice were characterized, on the one hand, by a somnolence severer than that seen with KO mice lacking HA alone, i.e., 1) significant decrease in W (during darkness and over 24h), sleep latencies and cortical EEG ratio between slow wave sleep and W; 2) unable to stay awake faced by a new environment. This somnolence was abolished by modafinil but not by H₃-receptor inverse agonists suggesting the absence of functional HA. On the other hand, these mice showed phenotypes characteristic of Ox KO mice, i.e., direct REM sleep onset (DREMs) and W deficit faced with a motor challenge, both being rescued by central Ox-A dosing. Finally, these mice displayed aggravated sleep fragmentation, obesity and also phenotypes never seen with the simple KO mice, i.e., EEG hypersynchronization and cataplexy, defined as sudden loss of muscle tone during W and characteristic of human narcolepsy.

Our data suggest that HA and Ox neurons exert a distinct but complementary and synergistic control on W, the amine being mainly responsible for cortical arousal and cognitive activities and the neuropeptide being more involved in behavioral activities. They could be co-responsible for narcolepsy: Ox deficiency is likely the direct cause of DREMs and cataplexy, whereas a decreased HA neurotransmission could account for the excessive somnolence seen in this disease and other sleep disorders.



O24

Major human histamine H₃ receptor isoforms display pharmacological differences

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Brain histamine is involved in the regulation of numerous functions of the central nervous system (CNS), with growing evidence for the involvement of the histamine H₃ receptor in arousal, cognition, locomotor activity, autonomic and vestibular functions, feeding and drinking, sexual behaviour, and analgesia. Moreover, H₃R specific ligands display beneficial effects in pre-clinical animal models of obesity, epilepsy, and cognitive diseases such as Alzheimer's disease and attention deficit hyperactivity disorder. As a result, H₃R antagonists are currently undergoing clinical trials (reviewed in [1]).

The aim of the present study is to pharmacologically characterize three common naturally occurring human histamine H₃ receptor (hH₃R) isoforms, hH₃R (445), hH₃R (365) and hH₃R (329) transiently expressed in HEK 293 cells as previously described [2] using [³H] GSK189254, a new high affinity and selective H₃ receptor inverse agonist [3]. Expression of each isoform was confirmed immunologically. These abundantly expressed human splice variants differ by a deletion of 80 and 116 amino acids in the intracellular loop 3, respectively and display a differential and overlapping expression pattern in human CNS.

In this report, we show the hH₃R (329) to have a 5-fold lower affinity 0.98 +/- 0.40 nM (n=4) for [³H] GSK189254 than either the hH₃R (445) 0.16 +/- 0.04 nM (n=4) or hH₃R (365) 0.24 +/- 0.07 nM (n=4). Interestingly, co-transfection (cDNA ratio 1:1) of the hH₃R (329) isoform had no effect on hH₃R (445) affinity 0.27 +/- 0.07 nM (n=4), but reduced by approximately 10-fold, [³H] GSK189254 affinity for hH₃R (365) 2.00 +/- 1.1 nM (n=5). Competition binding studies with the three isoforms either expressed alone or co-expressed show notable differences in pharmacology; data are currently being analysed. Previous reports have shown an 80 amino acid deletion (hH₃R (365)) in the intracellular loop 3 to confer higher affinity and potency for H₃R agonists and conversely lower affinity and potency for H₃R inverse agonists, when compared to the full length hH₃R (445) isoform [4]. We have confirmed this observation in our study.

These differences in H₃R pharmacology displayed by GSK189254 for the major human isoforms may be of importance for a detailed understanding of the clinical efficacy of H₃R ligands.

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O25

Histamine and Dopamine in Alcohol Addiction: Friends or Enemies?

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In this study, we have examined the involvement of histamine and H₃ receptor (H₃R) in ethanol-related behaviors in mice. Histidine decarboxylase knockout (HDC KO) mice displayed a weaker stimulatory response to acute ethanol than the wild type (WT) mice, whereas no genotype difference was found after ethanol administration in performance on the accelerating rotarod. The HDC KO mice showed stronger ethanol-induced reward than WT mice as measured by conditioned place preference (CPP) test, suggesting an inhibitory role for histamine in reward. In DBA/2J the H₃R antagonist ciproxifan had a tendency to enhance ethanol stimulation, but seemed to inhibit ethanol reward. In contrast, ciproxifan potentiated ethanol reward in 129/Sv mice. Differences in the sensitivity to ethanol and to H₃R drugs might underlie the opposite findings. Impepip, agonist of the H₃R, inhibited ethanol-stimulation but did not have a clear effect on ethanol CPP. Furthermore, in a drinking in the dark model ciproxifan decreased and impepip increased the consumption of ethanol. We also studied histamine metabolism and dopaminergic signaling in ethanol preferring (AA, Alko Alcohol) and non-preferring rats (ANA, Alko Non Alcohol). Previously, we found that AA rats have high histamine levels in the brain. Here we measured the expression of two histamine metabolizing enzymes, monoamine oxidase B and aldehyde dehydrogenase 1A1 in these rats but found no differences between the strains. We also found that the mRNA level of dopamine and cAMP regulated phosphoprotein (DARPP-32) was higher in striatal areas of AA rats as compared to ANA rats. Ethanol also activated DARPP-32 to a greater extent in AA rats than in ANA rats. Thus, our findings support a role for H₃Rs in the modulation of the ethanol stimulation, reward and ethanol drinking most likely via interaction with brain dopamine system. Data on AA rats imply that hypersensitized dopaminergic signaling might underlie the genetic preference to ethanol.



O26

Oleoylethanolamide and Brain Histamine Interact to Regulate Feeding Behaviour

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Oleoylethanolamide (OEA) is a satiety signal produced by the intestine after food consumption, and when given to rodents, it decreases significantly the amount of food eaten (1). Evidence indicates that histamine (HA) as well acts as a satiety signal, but nothing is known about the temporal/causal relationship between HA and OEA in controlling appetite or satiety. To learn whether OEA affects feeding behaviour via the HA system, mice lacking histidine decarboxylase (HDC-KO) and wild type (WT) littermates were food deprived for 12 h and then administered OEA (10mg/kg, i.p.) or saline. Food consumption was measured every 15 min for the 1st h and at increasing intervals for the following 24 h. As expected, OEA-treated WT mice eat significantly less than saline-treated WT mice. This effect was maintained for 24 h. However, the anorexiant effect of OEA was much attenuated in HDC-KO mice in the 1st hour and disappeared thereafter. To determine if OEA affects HA neurons' activity, OEA was administered systemically and HA release monitored using the microdialysis technique in freely moving rats. Male SD rats (250-280 g) were implanted with microdialysis probes, one in the TMN and the other in either the infralimbic cortex (IL) or the Nucleus accumbens (NAc), two HA projections areas involved in feeding behaviour. HA output, was measured in 15-min samples (flow rate 2- μ l/min) by HPLC-fluorimetric detection. I.p. injections of 10 mg/kg OEA induced a small but significant decrease of spontaneous HA release from the TMN (-30%; $p < 0.05$ ANOVA/Fisher's test; spont. release 0.052 ± 0.003 pmol/15min). To promote HA release in the TMN, IL and NAc we injected the H₃R antagonist ABT239 or bicuculline in the TMN. OEA attenuated both ABT239- and bicuculline-induced HA output increase from the TMN, the IL (spont. release 0.033 ± 0.002 pmol/15 min; $n=3$) and the NAc (spont. release 0.054 ± 0.003 pmol/15 min) by approximately 50%. The complex interactions between OEA and HA may have relevance in appetitive behaviors and satiety.

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O27

Effects Of Citalopram On Tail Suspension Test Require The Presence Of Neuronal Histamine

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5-HT increased histamine (HA) release from the anterior hypothalamus in anesthetized rats [1], and depolarized HA neurons [2]. Since the histaminergic tuberomammillary nucleus (TMN) receives 5-HT inputs from the raphé, we investigated whether SSRIs, mainstays in the treatment of depression, affect HA release. Citalopram, administered ip at 3-10 mg/Kg, increased significantly HA release from the TMN of SD male rats (b.w.: 250 g). HA was determined by microdialysis and HPLC-fluorometric detection. In another set of experiments, rats were implanted with one probe in the TMN, and one in the nucleus basalis magnocellularis or nucleus accumbens. Citalopram, added to Ringer, was perfused into the TMN at 2µl/min. HA was measured in 15-min fractions collected from both probes. Spontaneous HA release from all regions was stable, ranging 0.05-0.08 pmol/15min (N=27). TMN perfusion with citalopram (1-100 µM) for 60 min increased significantly HA release up to 100% of basal value from all areas (P<0.05, ANOVA/Fisher's test). Pretreatment with methysergide (10 µM), a 5-HT₂ receptor antagonist, abolished the effect of citalopram, suggesting that citalopram activates HA neurons by increasing the extracellular levels of endogenous 5-HT. To learn whether SSRI effects rest on the histaminergic system, we used the tail suspension test in normal and Histidine decarboxylase (HDC)-KO mice, which lack HA. Both citalopram (SSRI) and reboxetine (NSRI) reduced immobility in normal mice. Reboxetine was effective at reducing immobility also in HDC-KO mice, which, surprisingly, failed to respond to citalopram. Similar results were observed in normal mice acutely depleted of neuronal histamine through icv injection of alpha-fluoromethyl-histidine, a suicide inhibitor of HDC. These data show that HA plays important roles in mediating acute behavioural and neurochemical actions of citalopram.

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O28

Dopamine-induced arousal depends on the histaminergic system

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Dopaminergic neurons in the brain affect all stages of vigilance but the exact mechanisms of this regulation are poorly understood. The D₁-dopamine receptor mediates behavioural arousal, while D₂R activation induces biphasic effects: somnolence at low, waking at higher doses. Histaminergic neurons located in the tuberomammillary nucleus (TMN) of the posterior hypothalamus are wake-on pacemaker neurons controlling cortical arousal. We demonstrate variable expression of all 5 dopamine receptors in TMN neurons and increases in TMN neurons firing rate by quinpirole (D₂-like agonist), dopamine and L-DOPA. We tested the role of histamine in quinpirole-induced behavioural arousal with the help of histidine decarboxylase knockout mice. Quinpirole dose-dependently (1, 5, 15, 30 mg/kg) enhanced waking upon i.p. injection. The lowest dose increased waking and suppressed slow wave sleep (SWS) in WT, but not in HDC KO mice. Higher doses of quinpirole suppressed REM (rapid eye movement) sleep to a larger extent in KO than in WT mice. In conclusion, the histaminergic system is activated through D₂-like receptors and participates in dopamine-induced arousal.

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O29

Central H₁-receptor blockade increases sedation, but does not affect memory in healthy humans

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Animal literature suggests an important role for histamine in memory. In humans, this hypothesis has only been scarcely tested and results from studies that have addressed this are conflicting. In addition, some studies that administered H₁-antagonists to healthy volunteers detected some signs of impaired memory performance, but which may have been secondary to the sedative effects. The present study aimed to determine whether a centrally active antihistamine impairs memory performance and to dissociate such effects from sedation. Eighteen healthy volunteers received single oral doses of the antihistamine dexchlorpheniramine 4 mg, the benzodiazepine lorazepam 1 mg and placebo in 3-way, double blind, cross-over designed study. To measure memory functioning, subjects performed a 30 words-learning-task and an N-back task from which both electrophysiological (event related potentials) and behavioural measures (reaction time) were recorded. To measure sedation, subjective alertness and EEG frequency band power were recorded. Dexchlorpheniramine did not affect memory, but increased subjectively and objective measured sedation. The active control lorazepam impaired episodic- and working memory performance and increased sedation.



O30

Pharmacological Characterization of Oxime Agonists of the Histamine H₄ Receptor

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The histamine H₄ receptor (H₄R) has generated excitement as a potential target for the development of novel anti-inflammatory therapies. However, many of its physiological functions are still being uncovered and the development of new pharmacological tools is crucial to help facilitate this work. Previously, indole and benzimidazole piperazines have been described as potent and selective H₄R antagonists. Using this as a starting point we have identified new indole and benzimidazole oxime piperidines as ligands for the H₄R. These compounds have a high affinity for the human H₄R with K_i values ranging from 17-53 nM. They also have high to moderate affinity for the H₄R from mouse, rat, guinea pig and monkey, but poor affinity for the dog homologue. In addition to the high affinity for the H₄R, these compounds also exhibit excellent selectivity against other histamine receptors as well as many other receptor targets. These oxime ligands act as agonists of the human H₄R in transfected reporter systems, although the degree of agonism depends on the system utilized. Agonistic activity was also observed in human eosinophils as evidenced by their ability to induce a shape change in these cells, although the degree of agonism ranges from full agonist to partial agonist depending on the test conditions. In contrast to their activity at the human H₄R, all of the oxime compounds act as full agonists at the mouse receptor regardless of the test system including the ability to induce a calcium response in mouse bone-marrow derived mast cells. Finally the most selective compound, JNJ 28610244, was shown to induce scratching in mice indicating that it can also function as an agonist *in vivo*.



O31

2-Amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine Derivatives as Ligands of Histamine H₄ Receptor.

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The histamine H₄ receptor (H₄R) is the last histamine receptor subtype discovered so far. It is preferentially expressed on the hematopoietic and immune cells (basophils, eosinophils, mast cells and dendritic T cells), suggesting the role of H₄R signaling pathway in the inflammatory and immunomodulatory processes. Therefore, the therapeutic potential of H₄R ligands could provide novel promising therapies for different immuno-based diseases [1].

In the group of substituted 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives some compounds have very recently been described in patent literature as potent modulators of H₄R [2]. In addition to this, an increase in affinity at H₄R has been observed with additional amino groups at the heteroaromatic ring of the structurally related 4-(4-methylpiperazin-1-yl)-pyrimidine derivatives [3].

Taking these structure-affinity relationships into account we have designed and synthesized a series of novel compounds combining the features of 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives and their pyrimidine analogs containing an aromatic amino group. 2-Amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives with different alkyl and aryl moieties substituted in the 6-position of the triazine moiety were obtained and then tested for their affinities at recombinant human H₄R transiently expressed in insect SF9 cells.

The evaluated series of compounds showed *in vitro* affinities in the micromolar and submicromolar concentration range. The K_i value for the most potent compound has been obtained at 203±65 nM. In conclusion of this study, it should be stated that further modifications of the considered structural compound classes could lead to maintenance and in some cases to an increased affinity at H₄R.

Acknowledgement: This work was partly supported by the Polish Ministry of Science and Higher Education Grants No: 594/N-COST/2009/0, K/ZDS/000727 and the COST Action BM0806.

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O32

Discovery and SAR of Pyrimidine Derived Histamine H₄ Receptor Antagonists

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The initial histamine H₄ receptor high throughput screening campaign identified a tricyclic pyrimidine series of low nanomolar inhibitors. Though potent, this series suffered from rapid *in vitro* metabolism in human and rodent assays as well as narrow SAR around the diamine component. During the hit to lead campaign, we used a series of internal and literature observations to drive the program toward the discovery of the 6-alkyl-2,4-diamino pyrimidines. Subsequent optimization led to the discovery of a potent thiophene substituted histamine H₄ antagonist, with high selectivity over the other histamine receptors. This histamine H₄ antagonist has excellent PK properties with high bioavailability, good exposure and a reasonable volume of distribution and demonstrates efficacy in animal models. This presentation will discuss the SAR of the 6-alkyl-2,4-diamino pyrimidines as well as the profiling of representative analogs in PK and *in vivo* efficacy models.



O33

Is it possible to increase hit rates in virtual screening by multiple focusing? Indexing chemicals for their H₄ receptor antagonism

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The most recently characterized H₄ histamine receptor (H₄R) is a promising target in the therapy of inflammatory diseases and disorders of the immune system. Selective antagonists of H₄ receptor may have therapeutic significance in treating allergy, inflammation, autoimmune disorders, pain conditions and possibly cancer. The utility of multiple *in-silico* techniques to increase the hit rate in virtual screening experiments, seeking new H₄R antagonists, is discussed herein.

Indexing via chemoinformatics tool: by utilizing the ILE technology¹, a “molecular activity index” (MAI) is produced on the basis of optimized differences between H₄-antagonists and non-active molecules. We performed virtual high throughput screening for large database of chemicals (e.g. ZINC database that contain more than 8 million commercially-available compounds, <http://zinc.docking.org/>) and pick highly anti-inflammatory “focused library”. Pharmacophore filtering: pharmacophoric features generated from H₄ receptor modeled structure and H₄R antagonists are applied in a second stage for further processing focused library and prior the last stage of filtering via docking.

Docking: since the H₄ receptor is a member of the G-protein coupled receptors (GPCRs) family its 3-D structure prediction is a big challenge due to the limited availability of resolved GPCRs' structures. The X-ray structures have been solved for only five such proteins. The identity between hH₄R and the potential templates is less than 35%. We conducted analyses of a large database of human G-protein coupled receptors that are members of rhodopsin like family in order to optimize the available crystal structures for molecular modeling of hGPCRs². On the basis of our findings, specific parts from the trans-membrane domains of the reference receptor helices were proposed as appropriate template for constructing models of other GPCRs, while other residues require other techniques for their remodeling and refinement. Side chains³ and loops⁴ are predicted with ISE-based tools. The predicted H₄R was refined by all-atom molecular dynamics simulations performed either in water or in membrane-like environment and utilized for docking purposes to filter potentially H₄R antagonists which pass the molecular activity index threshold and the pharmacophore.

Multiple focusing with chemoinformatics, pharmacophore and docking may increase enrichment rates in H₄R antagonist model compared to utility of one technique alone. Certainly, the described approach could have important impact on decision making in the fields of screening molecules for their biological activity, lead identification and lead optimization.

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O34

Fragment optimization leading to H₄ receptor ligands with differential pharmacology

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Soon after the discovery of the histamine H₄ receptor in the human genome, this new G-protein coupled receptor has been identified as a new potential therapeutic target for various inflammatory conditions, including e.g. allergic rhinitis and pruritis. To establish the full potential of H₄ receptor ligands as future therapeutics, several labs, including ours have been searching for new selective agonists and antagonists.

Following a fragment-based hit finding program, we have identified various small scaffolds with (sub)micromolar affinity for the human H₄ receptor, as measured by [³H]histamine radioligand binding studies. Medicinal chemistry efforts allowed us to optimize the fragments rapidly to various quinoxaline-, aminopyrimidine-, and quinazoline analogues with low nanomolar affinity at the human H₄ receptor. Selected representatives of the various scaffolds were also tested against mouse and rat H₄ receptors in both binding and functional experiments. Interestingly, several of the compounds displayed significant species differences with respect to both binding and the functional activities. These *in vitro* differential pharmacological effects could not always be directly translated to the observed *in vivo* effects in a model of inflammation (rat carageenan induced paw edema).

Our data suggest that high affinity H₄ receptor ligands with tunable functional activities can be developed, but that one should also test both binding and functional activities at non-human orthologs for a good interpretation of *in vivo* effectiveness.



O35

Acidic elements in histamine H₃ receptor ligands

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Human histamine H₃ receptors (*hH₃R*) in the central nervous system (CNS) modulate synthesis and liberation of histamine and various other neurotransmitters, thereby acting as auto- and heteroreceptors, respectively. Hence, the *hH₃R* plays a major role in controlling the release of numerous neurotransmitters in brain compartments with co-localized neurons and affects functions like vigilance, learning, attention or feeding behaviour.[1]

The *hH₃R* targeting protein domain and its corresponding antagonist pharmacophore are well investigated. Actually, most *hH₃R* antagonists contain a basic moiety, which is coupled *via* an alkyl spacer to a central core. This can be substituted with a variety of basic, polar or hydrophilic moieties of different sizes. A second basic moiety usually boosts potency but also covers the risk of central accumulation and the respective side effects. Starting from this so-called class of diamine-based *hH₃R* ligands [2] we prepared acid containing compounds by coupling acidic moieties of different estimated pK_a values (2.2–7.8) to an established *hH₃R* pharmacophore. In a displacement assay, the majority of compounds exhibited affinities in the low nanomolar concentration range ($pK_i = 7.3$ –8.7) explicitly proving that the *hH₃R* accepts these acidic pharmacophores and probably contains a basic area within the binding pocket. Thus, the so far accepted doctrine among *hH₃R* researchers is broadened offering new possibilities for the development of *hH₃R* antagonists.

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O36

***para-t*-Pentylphenoxyalkyl Piperidine Derivatives as Potent Histamine H₃ Receptor Ligands**

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Histamine H₃ receptors are constitutively active G-protein coupled receptors mostly expressed in CNS, described as presynaptically located autoreceptors as well as heteroreceptors. Interaction with these receptors results in modulation of histamine levels as well as that of other neurotransmitters such as ACh, NA, 5-HT etc. Therefore blockade of these receptors could be useful in the treatment of different CNS disorders [1].

First known histamine H₃ receptor antagonists contained an imidazole group, which potentially may be responsible for some side effects due to its interaction with cytochrome P₄₅₀. The first successful imidazole replacement has been obtained with piperidine moieties. In the proposed general pharmacophore for histamine H₃ receptor antagonists, the heterocyclic residue should be connected via an aliphatic linker with a polar moiety, connected itself by another linker to a lipophilic residue.

As a template for our research we used compounds DL-76 (*para-t*-butyl), DL-77 (*para-t*-pentyl) derivatives previously described in our group with related side-branched moieties (hH₃ K_i = 22, 8.4nM respectively) [2]. Paying attention to our previous investigations [2], and the results described in the literature, we obtained *p-t*-pentylphenoxyalkyl derivatives with ascending alkyl chain length (5-8 carbons), to evaluate the influence of the elongated alkyl spacer on histamine H₃ receptor binding properties.

The novel compounds were evaluated for histamine H₃ receptor *in vitro* affinity in the [¹²⁵I]iodoproxyfan binding assay to histamine hH₃ receptor stably expressed in HEK-293 cells. Presented compounds show high affinities (hH₃ K_i values in the range of 8.8-325.9 nM). Computational approaches, using Schrodinger MacroModel 9.7, allowed the *in silico* visualization of binding to histamine H₃ receptor model described by Levoine *et al.* [3].

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O37

Systems Biology on histamine H₄ receptor activity

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Most corporal histamine remains stored in the granules of mast cells and basophils and is released mainly as an immunological response. The autocrine effect of histamine in both cell lines mediates chemotaxis, calcium mobilization and protein kinase cascades.

The *ab initio* study of the processes involved in the activation of the GPCR histamine H₄ receptor [1], could be an arduous task in view of the assumed complexity of the cascade of reactions that normally occur in these cells during activation. A computational systemic study of H₄R functional relationships together with proteomics experiments is ongoing with the goal of unraveling the biological mechanisms triggered by H₄R activation.

We have compared the proteomes of the human mast cell leukemia cell line HMC1 untreated against the cells treated 24 hours with histamine 100 nM, enough to activate H₄R and far from the dissociation constant of the other known histamine receptors, and with histamine 100 nM together with the H₄R antagonist JNJ7777120 100 nM.

The modelling of functional genetic interaction network for H₄R, based on the existing information about protein-protein associations reveals the lack of knowledge about this system. We are determining new binary interactions by the integration of different protein association prediction methods [2] to model gene networks functionally associated to the datasets identified by our proteomics approach. Our H₄R network model will be used to address the systematic functional characterization of the proteomic data and to optimize selection of novel targets involved in the H₄R activation system.

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O38

Indexing drugs for their cardio-toxicity

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The large number of non-antiarrhythmic drugs that prolong QT interval is rising, including antihistamines, antipsychotics and antibacterials. Recently the issue of drug-induced long QT syndrome (LQTS) has become one of the key reasons for which some drugs fail to enter market, while others have been withdrawn from the market. Early identification of chemical entities causing LQTS is of extreme importance relevant to the production of safer drugs as well as to the direct reduction of attrition rate in drug development. We will describe new methods for indexing drugs for their cardio-toxicity and h-ERG liability (Iterative Stochastic Elimination¹ and Intelligent Learning Engine²). The analyses and modeling have been carried out on two systems: drugs inducing LQTS/cardio-safe drugs and h-ERG binders/non-binders³⁻⁴. The accuracy of the new techniques is compared to the state-of-art classification techniques. The proposed models could be employed to infer cardio-toxicity or -safety for current and potential drugs. Certainly, it will also have important impact on decision making in the fields of screening molecules for drug development, biological activity, and other applications as well.

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O39

Application of the bivalent ligand approach to acylguanidines resulted in highly potent and selective histamine H₂ receptor agonists

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N^G-Acylated hetarylpropylguanidines represent a new class of potent histamine H₂ receptor (H₂R) agonists [1, 2] with improved pharmacokinetic properties due to substantially (by 4-5 orders of magnitude) reduced basicity compared to the corresponding N^G-alkylated analogues. In continuation of this work we explored the bivalent ligand approach by analogy with the concept described by Portoghese for opioid receptors [3]. The aim of this project was to study structure-activity relationships and to develop pharmacological tools for the investigation of hypothetical dimeric histamine H₂Rs.

The synthesized compounds were investigated for H₂R agonism at the isolated guinea pig (gp) right atrium and in a steady-state GTPase assay using human and guinea pig H₂R fusion proteins expressed in Sf9 insect cells (H₂R-G_q). Moreover, the histamine receptor selectivity profile (H₂R vs. H₁R, H₃R, H₄R) was determined (GTPase assays).

The bivalent acylguanidines turned out to be highly potent and selective H₂R agonists. Most strikingly, the combination of two hetarylpropylguanidines with octanedioyl to decanedioyl spacers led to the most potent H₂R agonists described so far (up to 4000-fold more potent than histamine). Due to insufficient spacer length of the most potent ligands for simultaneous interaction with both binding pockets of a hypothetical H₂R dimer the tremendous gain in potency is presumably due to the interaction with an additional binding site at the same receptor molecule. Furthermore, investigations on mutant H₂Rs confirmed the key role of non-conserved Tyr-17 and Asp-271 in TM1 and TM7 in the gpH₂R for species-selective H₂R activation and suggest that the second extracellular (E2) loop does not participate in direct ligand-receptor interactions.

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Poster sessions Abstracts



P1

Histaminergic modulation of striatal function

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The basal ganglia are a group of brain nuclei essential for the control of movement, as well as a variety of other functions. Dysfunction of these nuclei is implicated in ADHD, Huntington's and most famously Parkinson's disease. The striatum is the primary input nucleus of the basal ganglia which receives major excitatory inputs from the cortex and thalamus. The predominant cell type of the striatum is the GABAergic medium spiny (MSN) projection neuron. MSNs are divided into so called 'direct pathway MSNs' that project directly to the output nuclei of the basal ganglia and express the D1 subtype of the dopamine receptor and 'indirect pathway MSNs' which express the D2 subtype. The activity of neurons in the striatum is strongly controlled by neuromodulators (e.g. dopamine, serotonin and histamine).

We set out to investigate the role of histamine in the modulation of the excitatory input to the striatum derived from the cortex and thalamus, and inhibitory inputs derived from MSNs and striatal interneurons. To this end we performed whole-cell voltage-clamp recordings of D1-expressing and D2-expressing MSNs in *Drd1-EGFP* and *Drd2-EGFP* transgenic mice. We investigated the effect of bath-applied histamine (10 μ M) in conjunction with selective histamine receptor antagonists on responses evoked by cortical or local stimulation. The D1 and D2 phenotype of the MSNs was subsequently confirmed by immunocytochemistry for EGFP.

We found that both cortical and inhibitory inputs to MSNs were negatively modulated by bath applied histamine. Co-application of histamine and the H3 receptor antagonist, thioperamide (10 μ M), blocked this negative modulation.

These results support the conclusion that the histaminergic innervation of the striatum is involved in the negative regulation of cortical excitatory and striatal inhibitory inputs via the H3 receptor.

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P2

Pharmacological properties and precognitive effects of ABT-288, a potent and selective histamine H₃ receptor antagonist.

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The histamine H₃ receptor (H₃R) is an attractive target for the treatment of cognitive disorders since blockade of this receptor enhances central neurotransmitter release. The *in vitro* and *in vivo* pharmacological properties of the H₃R antagonist ABT-288 were profiled in our laboratories. ABT-288 is a potent and selective competitive antagonist of human and rat H₃Rs (K_is = 1.9 and 8.2 nM, respectively) that enhances the release of histamine, acetylcholine, and dopamine in rat prefrontal cortex. In cognition studies, ABT-288 improved acquisition of a five-trial, inhibitory avoidance test in rat pups (0.001–0.03 mg/kg), social memory in adult rats (0.03–0.1 mg/kg), and spatial learning and reference memory in a rat water maze test (0.1–1.0 mg/kg). *In vivo* rat brain H₃R receptor occupancy of ABT-288 corresponded to efficacious doses and exposure levels in behavioral models. ABT-288 demonstrates a number of favorable attributes including good pharmacokinetics and oral bioavailability, with a wide CNS and cardiovascular safety margin. Thus, ABT-288 is a selective and potent H₃R antagonist with drug-like properties and broad efficacy across animal cognition models suggesting potential clinical efficacy for cognitive disorders such as Alzheimer's disease, ADHD, and cognitive deficits of schizophrenia.



P3

The Effects of Bilastine 20 mg and 40 mg, and Hydroxyzine 50 mg on Actual Driving Performance

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Bilastine is a new second generation H₁ antagonist used in the treatment of allergic rhinitis and chronic urticaria. Although bilastine has been demonstrated to produce no or little performance impairment on laboratory tests, it cannot be excluded that the drug produces performance impairments in real life performance such as driving.

The present study was designed to assess the effects of two doses of bilastine (20 and 40 mg) on actual driving after single and repeated dosing. Hydroxyzine 50 mg, a highly sedative antihistamine, was included as a control treatment to demonstrate the sensitivity of the performance measures.

The study consisted of 22 healthy volunteers (11 male, 11 female; 21 to 45 years) and was conducted according to a randomized, double blind, 4 way cross-over design. Participants were treated with once daily doses for 8 consecutive days. On day 1 and 8 of each treatment period participants conducted the actual highway driving test. In this standardized driving test participants operate a specially instrumented automobile over a 100 km (62 mile) primary highway circuit. The participants task is to maintain a constant speed of 95 km/hour (59 miles/hour), and a steady lateral position between the delineated boundaries of the right (slower) traffic lane. A licensed driving instructor accompanies the participant.

Contrasting each drug with the placebo showed that weaving, the primary measure of the highway driving test, was significantly increased after treatment with hydroxyzine on both day 1 ($F_{3,15}=14.29$; $p=0.000$) and day 8 ($F_{3,15}=4.23$; $p<.05$), but not after treatment with either bilastine 20 mg or 40 mg.

In conclusion, bilastine 20 mg and 40 mg had no significant impairing effects on driving performance. However, hydroxyzine 50 mg produced impairing effects on driving performance, with no development of full tolerance after one week of treatment.



P4

Histamine H₂ receptor immunoreactivity in the mouse brain

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The tuberomamillary histamine neurons regulate brain functions through 3 G protein-coupled receptors expressed abundantly in many nuclei. The H₂ receptor activation excites or facilitates excitation in different parts of the brain. Since the mRNA distribution does not show where along the neuronal projections the receptor protein resides, we studied the immunolocalization of H₂R in mouse brain using an antibody generated against a synthetic peptide corresponding to a portion of the C-terminal part of the receptor.

The antiserum stained neurons in e.g. cerebral cortex, neostriatum, hippocampus, dentate gyrus, olfactory bulb, septum and pons. The staining was consistent with cell surface labelling of cell bodies and proximal dendrites of cells with characteristic neuronal morphology in the cortex. This pattern is in agreement with a characteristic postsynaptic receptor function. The immunoreactivity was blocked completely with a peptide conjugate suggesting specificity of the staining. Two distinct bands were seen in Western blot analysis of different brain regions of the mouse. Transcription expression level of different region of the brain was confirmed with quantitative PCR. Distribution of H₂ receptor immunoreactive neurons was in general agreement with data on H₂ receptor mRNA distribution in mouse brain, which is more uniform than in some other species.

The antibody will allow identification of neuronal and possibly non-neuronal cell types which express H₂ receptor in the brain and other tissues, and studies on dynamic regulation of H₂ receptor in different neurons.



P5

The Effects of Properties of Food on Amygdalar Histamine Release in Rats.

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The histaminergic system is known to be involved in feeding behavior, and our previous study indicated that the histaminergic system is activated by feeding behavior. We also showed that gustatory information influences the histaminergic system, and that palatability of taste solution affects its activity. When animals eat food, the oral cavity receives a variety of sensory information from food, such as taste and texture. This information is conveyed to the higher brain regions, and plays an important role in the regulation of feeding behavior. However, the effects of the properties of food, such as taste and texture of food, on the activity of the histaminergic system are poorly understood. Therefore, in the present study, we examined the effect of the properties of food on histamine release in the central nucleus of amygdala by *in vivo* microdialysis using freely moving rats.

We used normal, soft, and sweet pellets: normal and soft pellets are made with similar ingredients but have different degrees of hardness (normal > soft), and sweet pellets have the same hardness of normal pellets and contain 3% sucrose per weight. In the food choice test, when the rats were presented with two types of pellets, soft pellets and normal pellets or sweet pellets and normal pellets, they preferred soft pellets to normal pellets, sweet pellets to normal pellets, respectively. This observation indicates that the hardness and taste of food plays a role in the selection and ingestion of food.

In the microdialysis study, histamine release was significantly increased in the rat fed with normal pellets in accordance with our previous study. By contrast, histamine release was not enhanced both in soft pellets-fed rats and in sweet pellets-fed rats. There were no significant differences between the amounts of normal, soft and sweet pellets intake of experimental period. From these findings, the amygdalar histaminergic system is suggested to be modulated by the properties of food.



P6

Species-directed immunological probes for the H₄ histamine receptor: evidence for multiple roles for the H₄R

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The histamine H₄ receptor (H₄R) is the latest receptor identified belonging to the histamine receptor subfamily of G protein coupled receptors (GPCR). Until recently the H₄ receptor was thought to be largely expressed upon haematopoietic cells, and therefore a potential major new target for inflammatory disorders [1]. It was not until recently that the H₄R was shown to be functionally expressed in the central nervous system (CNS) [2]. Recent studies have shown selective H₄R antagonists display antinociceptive and antipruritic properties [3]. The rat and mouse H₄R shares low overall homology with the human H₄R 69% and 68%, respectively. Furthermore, H₄ receptor ligands have been shown to display varying *in vitro* pharmacologies across the different H₄R species orthologues [5]. In all species tested thus far the full length H₄ receptor encodes a polypeptide of 390/391 amino acids (predicted polypeptide approx M_r 40,000). Herein, we have attempted to develop the first panel of species-specific immunological probes for the H₄R.

In order to define the importance of H₄ receptor species heterogeneity, specific-directed immunological probes are required. Antibodies were raised against peptide sequences unique to the mouse, rat and human H₄ receptor. Initially, we generated an anti-human H₄ specific antibody raised to Cys-IKKQPLPSQHSRSVSS, which identified major monomeric and dimeric species (M_r 37,000 and M_r 74,000) in recombinant cells and (M_r 34,000 and M_r 68,000) in native tissue [4]. This antibody also recognized the rat and mouse H₄ receptor, despite the modest sequence similarity, on grehlin-positive endocrine cells in the rat GI tract [7], mouse salivary gland acinar cells [this meeting], and mouse brain and spinal cord [3, 6, unpublished], respectively. Due to the antigen sequence differences, the levels of expression detected are likely to be underestimates. Therefore, we have generated an anti-mouse H₄ specific antibody raised to Cys-VTKQPALSQNQSVSS, which identified major monomeric and dimeric species (M_r 44,000, M_r 74,000, M_r 109,000) in recombinant cells and (M_r 30,000 and M_r 66,000) in native tissue [6]. We are currently developing and validating a novel anti-rat H₄ specific antibody to complete the panel.

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P7

Immunological probes for human H₃ histamine receptor isoforms

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The existence of multiple H₃ receptor isoform mRNAs has opened up possibilities to account for the pharmacological heterogeneity in H₃ receptors, within and across species, which has long been recognised. The reasons for this heterogeneity are complex and not fully understood. While the full length H₃ receptor isoform is found in most abundance in the CNS in all species studied so far, there is regional variation in the distribution of the different isoform mRNAs. This has given rise to speculation that H₃ heterogeneity could underlie different activities and functions of H₃ receptors in specific brain areas. Furthermore, we have growing evidence that heteroligomerisation of H₃ isoforms may occur and yield a novel regulatory mechanism [1]. In order to define the importance of human H₃ receptor heterogeneity, specific isoform specific immunological probes are required. Our laboratory has developed the first panel of anti-human H₃ receptor isoform-directed antibodies. Two antibodies (termed anti-pan hH₃R) were raised against human H₃ receptor sequences common to most human and rodent isoforms: anti-hH₃ (346-358) and anti-hH₃ (175-187), respectively [2, 3, Hann et al., unpublished]. Recently, we have generated an anti-hH₃ (445) specific antibody [1], an anti-hH₃ (329) specific antibody, and an anti-hH₃ (200) (isoform 5). The human H₃(365) has an 80 amino acid deletion within the third intracellular loop which results in the isoform displaying interesting pharmacological differences compared to the full length hH₃R(445), higher affinity and potency for H₃R agonists and conversely lower affinity and potency for H₃R inverse agonists [5, Lethbridge *et al* this meeting]. We have generated a novel anti-hH₃ (365/445) antibody raised to peptide sequence EAMPLHRKYAKALA, which identified a major specific species (M_r 35,000 and M_r 70,000) in HEK293 cells transfected with human H₃R(365) isoform on immunoblotting, which was blocked by preincubation with the respective peptide. The size of this immunoreactive species corresponds to monomeric and dimeric hH₃(365). The ability to distinguish between the human H₃R isoforms will provide insight into their functional relevance especially in the CNS where the H₃R is dominantly expressed. This will aid the way for the development of isoform specific ligands (see Lethbridge et al., this meeting), and limit potential side effects associated with general H₃R ligands. These antibodies have been successfully utilized in human postmortem brain tissue and display differential expression patterns (Ling *et al.*, this meeting).

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P8

Identification of H₃ and H₄ Histamine Receptors on Normal and Cystic Fibrosis Epithelial Cells

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Abnormal chloride transport is a characteristic feature of cystic fibrosis (CF). Histamine has been shown to activate chloride efflux in other cell types, including epithelial cells. This may prove beneficial in CF. The primary aim of the current study is to investigate the presence of histamine receptors on normal and cystic fibrosis bronchial epithelial cells.

Using HBE and CFBE cell lines and primary human nasal epithelial cells from controls (HNEC) and patients with cystic fibrosis (CFNEC), the presence of both H₃ and H₄ histamine receptors was investigated using Western Blot and immunocytochemical staining and a range of previously validated anti-hH₃ and anti-hH₄ selective antibodies [1, 2]. Histamine induced cytokine release was measured by IL-8 ELISA.

Both H₃ and H₄ receptor proteins were detected in all cell types and this was confirmed by immunocytochemical staining. Addition of histamine to HBE and CFBE cells increased IL-8 release.

The presence of histamine receptors on CF epithelial cells may provide an important route to activating chloride transport in these cells.

1. KE Cannon *et al.* (2006) *Pain* 129, 76-92
2. R van Rijn*, PL Chazot* *et al.* (2006) *Mol. Pharmacol.* **70**, 604-615

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P9

Histamine H₄ receptor mediated suppression of IL-12 family cytokines

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The histamine H₄ receptor (H₄R) is primarily expressed on hematopoietic cells. The distinct expression profile indicates the H₄R as a promising target for immune disorders in which these cells are involved. The present study was performed to determine effects of the H₄R on dendritic cell production of IL-12, IL-23 and IL-27 belonging to the so called IL-12 family due to similarities in their receptor subunit composition. Cytokines of the IL-12 family are involved in the initiation and orchestration of Th1 responses. The modulation of IL-12 response may have an impact on Th2-Th1 skewing of chronic allergic diseases. Murine bone marrow derived dendritic cells (DC) were generated from wild type (WT) and H₄R knockout mice (obtained from Lexicon Genetics, Inc., through Johnson & Johnson Pharmaceutical Research & Development, L.L.C). After nine days of cultivation, DC were stimulated with various *toll like* agonists. Cytokines were determined 24 hours later in the supernatants of DC generated from WT and H₄R knockout (H₄R KO) mice by means of ELISAs. There was an increase of TNF α , IL-6, IL-12, IL-23 and IL-27 in supernatants of WT as well as H₄R KO stimulated DC. The increase of TNF α , IL-6 did not differ between WT and H₄R KO DC, whereas there was a constant higher secretion of IL-12, IL-23 and IL-27 in H₄R KO DC compared to WT DC indicating, that the H₄R plays a substantial role for the modulation of IL-12 related cytokines. However, when DC were pre-incubated with histamine (1 mmol/l), there was a decrease of IL-12 concentration in the supernatants from WT DC as well as H₄R KO DC. This indicates that the H₄R is not solely responsible for the modulation of IL-12 secretion, which is in accordance with findings in human monocyte derived dendritic cells, where an additional involvement of H₁R and H₂R in attenuating IL-12 secretion is postulated.



P10

Role of H₄ Receptor in Histamine-induced Inhibition of Human Breast Cancer Cell Proliferation

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Histamine plays a critical role in growth regulation and differentiation during mammary gland development. Histamine H₁, H₂, H₃ and H₄ (H₄R) receptors are expressed in different normal and malignant cell lines, and also in benign lesions and tumours derived from human mammary gland. We have reported that histamine regulates differentially signalling processes in normal and malignant breast cells. The aim of the present work was to investigate the biological responses triggered by histamine through the H₄R in two breast cancer cell lines with different malignant characteristics. For that purpose in MDA-MB-231 [estrogen receptor (ER) α -] and MCF-7 (ER α +) breast cancer cell lines, we evaluated cell proliferation by the clonogenic assay, cell count and the incorporation of BrdU using H₄R agonists [Clobenpropit (Clob) and VUF8430 (VUF)] and antagonists. Furthermore, cell senescence and differentiation were determined by β -galactosidase enzyme assay and lipid accumulation using Nile red staining and flow cytometry, respectively. Apoptosis was studied by Annexin-V staining and flow cytometry, and TUNEL assay while the mitochondrial transmembrane potential ($\Delta\psi$ m) was investigated by DiOC6 staining and flow cytometry. In MDA-MB-231 cells, Clob and VUF treatments significantly decreased proliferation to 45.5 \pm 14.8% and to 76.7 \pm 5.3%, respectively. This effect was associated to a reduction in BrdU incorporation, an augmentation in Annexin-V and TUNEL positive cells ($P < 0.01$), a decrease in the $\Delta\psi$ m (81.5%) and a 2.5 fold increase in the cell senescence. In MCF-7 cells, H₄R agonists inhibited proliferation by 50% increasing the exponential doubling time from 32.6 h to 46.4 h and 47.3 h in Clob and VUF treated cells, respectively. The negative effect on proliferation was related to an increase in Annexin-V and TUNEL positive cells ($P < 0.01$), a decrease in the $\Delta\psi$ m (59.5%) and a 2-fold increase in cell senescence. We conclude that the H₄R is involved in the regulation of breast cancer cell proliferation, apoptosis and senescence.



P11

Histamine H₄ Receptor in Human Melanoma Cells and Tissues.

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Human malignant melanoma is a highly metastatic cancer that is markedly resistant to conventional therapy. The expression of the histamine H₁, H₂, and H₃ receptors in melanoma cell lines and a role for histamine in melanoma cell growth has been reported. We have previously demonstrated the presence of histamine H₄ receptor (H₄R) in WM35 primary human melanoma cells. In this cell line histamine inhibits proliferation and migration, and induces differentiation and senescence through the activation of H₄R. The aim of this work was to investigate the presence of H₄R in M1/15 cells (highly metastatic human melanoma cell line) and its associated biological processes. In addition, we evaluated the presence of the H₄R in human melanoma biopsies. The expression of H₄R in M1/15 cells was analyzed by RT-PCR, and Western blot while in biopsies it was determined by immunohistochemistry. To characterize the biological responses, we evaluated cell proliferation by the clonogenic assay and the incorporation of BrdU. Furthermore, cell senescence and differentiation were determined by β -galactosidase enzyme assay and dopa oxidase activity, respectively. Apoptosis was studied by Annexin-V staining and flow cytometry. Results indicate that M1/15 cells express H₄R at the mRNA and protein level. By using histamine agonists and antagonists we showed that the inhibitory effect of histamine on proliferation is in part mediated through the stimulation of the H₄R (VUF 8430 IC₅₀=4,8 μ M). The decrease in proliferation was associated with an induction of cell senescence and an increase in melanogenesis that is a differentiation marker of these cells. In addition, we demonstrated that the H₄R was detected in the 71% of the melanomas that also exhibited a high level of histamine content. On the basis of the presented results, we conclude that M1/15 melanoma cells express H₄R which is involved in histamine-mediated cell proliferation, senescence and differentiation. Accordingly, H₄R is present in highly invasive human melanoma lesions indicating a potential therapeutic application of H₄R ligands.



P12

Histamine Regulates MDA MB 231 Breast Cancer Cell Line Invasive Potential.

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We have previously demonstrated that MDA MB 231, a metastatic breast cancer cell line, expresses the four known types of histamine receptors which differentially regulate cell proliferation. We have also determined that histamine increases MDA MB 231 cells intrinsic sensibility to ionizing radiation.

The aim of the present study was to investigate the role of histamine in cellular events involved in metastatic capacity, such as expression and activity of matrix metalloproteinases (MMPs), cell migration and invasion. The possible interaction between histamine treatment and irradiation was also evaluated since ionizing radiation may affect metastatic competence depending upon cell type and irradiation characteristics.

Histamine in concentration over 10 uM decreased MMP2 and MMP9 expression assessed by RT-PCR and cytochemistry as well as enzymatic activity determined by zymography. This effect was mimicked by H₂ agonist amthamine, while an opposite action was observed when H₄ agonist VUF 8430 was employed. Experiments using transwell systems demonstrated that 1uM histamine increases cell migration, though concentrations higher than 10 uM decreased migration. Also, wound healing assays showed that cell motility was significantly augmented via H₄ receptors, though diminished through H₂ receptors. In addition, assays employing matrigel coated tranwells showed that histamine enhanced MDA MB 231 cells invasion ability via H₄ receptors.

Cells irradiated with a 2 gray Dose showed a rise in metalloproteinases activity and cell motility compared to control cells; however this effect was counteracted by histamine treatment.

In summary, histamine differentially regulates expression and activity of matrix metalloproteinases, cell migration and invasiveness through H₂ and H₄ receptors in MDA MB 231 cells. Results also suggest that histamine could improve radiotherapy efficacy regarding the potential development of metastases.



P13

Effects of histamine H₄ receptor ligands on rodent models of acute inflammation

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Previous data from our laboratories have demonstrated that histamine H₄ receptor (H₄R) antagonists have anti-inflammatory effects in a rat paw edema model of acute inflammation (Coruzzi et al., 2007). In the present study the effects of the selective H₄R agonists VUF8430 (Kitbunnadaj et al., 2004) and VUF10460 were investigated in the same model after different route of administrations. Whereas subplantar injection of VUF10460 (20 mM) into the rat paw was without effect, VUF8430 (20 mM locally) induced a dose-dependent increase in paw volume, which, however, was not related to H₄R activation. VUF8430 (30-100 mg/kg) and VUF10460 (10-30 mg/kg) significantly reduced (from 30 to 95% inhibition) carrageenan-induced edema, when administered subcutaneously. In the ear mouse model, local administration of croton oil (20 µl/ear) induced an increase in ear weight, which was significantly reduced by dexamethasone, administered either locally (0.05 mg/ear) or subcutaneously (2 mg/kg). By contrast the reference H₄R antagonist JNJ7777120 (10-30 mg/kg sc or 0.03 mg/ear) was without effect. VUF10460 (30 mg/kg sc) and VUF8430 (100 mg/kg sc) significantly reduced ear mouse edema induced by croton oil; both compounds were ineffective when administered locally. These data indicate that selective H₄R agonists do not possess pro-inflammatory effects in two models of acute inflammation when injected locally. However, they display anti-inflammatory effects when administered systemically against different models of acute inflammation. Finally, these results confirm that H₄R ligands may display different pharmacological properties, depending on the experimental assay.

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Kitbunnadaj R et al., J Med Chem 2004 ; 47:2414-7

P14

Expression Patterns Of Histamine Receptors In The $G\alpha i2$ -Deficient Mouse Model Of Colitis.

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Crohn's disease (CD) and Ulcerative colitis (UC) are the idiopathic forms of inflammatory bowel disease (IBD) in humans. Many studies on IBD have been performed in different knockout mice as animal models of colitis; mice deficient in the G protein $\alpha i2$ subunit, $G\alpha i2^{-/-}$ mice, being one of them. They develop a disease similar to UC including development of colon cancer (Rudolph U et al. Nat Genet 1995;10:143-50) with a T helper 1 dominated immune response (Hörnquist CE et al. J Immunol. 1997; 158:1068-77). The most recently described histamine receptor; the histamine 4 receptor (H_4R) is expressed on various immune cells. The selective H_4 receptor antagonists have been shown to reduce pathology in TNBS-induced colitis in rats (Varga C et al. Eur J Pharmacol 2005; 522:130-8). Thus the H_4R may play a role in IBD development especially as increased amounts of histamine in the gut lumen of IBD patients have been reported. We have analysed expression of histamine receptors H_1R , H_2R , and H_4R in mucosal tissues from $G\alpha i2^{-/-}$ mice in different stages of colitis by real-time reverse transcription-polymerase chain reaction (RT-PCR). We found that in colon, there were 3-fold reductions of both H_4R and H_2R expression in precolitic mice compared to wild type mice ($P<0.05$). The H_4R levels were increased with the colitis progression, as they were markedly elevated from pre colitis to late colitis ($P<0.01$). However, the H_2R expression was not changed by the colitis development, as it remained the same in different stages of colitis. The H_1R expression in $G\alpha i2^{-/-}$ mice remained the same as in the wild type mice. In the small intestine, our preliminary data demonstrate that the H_4R levels were slightly increased in early and late colitis mice compared to wild type mice, whereas there were no difference between precolitic and wild type mice. However, there were no changes in H_1R or H_2R levels between wild type mice and $G\alpha i2^{-/-}$ mice.



P15

The immunogenic role of microvesicles in asthmatic and healthy pregnant

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Asthma is the most common, potentially serious medical problem during pregnancy. It's prevalence is increasing, today it is between 4-7% worldwide among pregnant women. The increased risk of complications can lead to preeclampsia, preterm delivery and low birth weight. The severity of asthma is changing during gestation, often in an unpredictable way.

It is a relatively recent finding that microvesicles (MV) represent a pivotal role in information transfer between cells. MVs are membrane covered small particles, derived from various cell types. Their possible diverse origin includes, among others, blood cells and trophoblast cells, so MVs are involved in cell to cell crosstalk as cellular effectors. Successful pregnancy requires a series of interactions between the maternal immune system and the implanted fetus.

Our group has started to search for the role of MVs in the third trimester of pregnancy. We compared the plasmas of four groups: healthy and asthmatic pregnant women and healthy and asthmatic non-pregnant women. We found significant differences in the quantity of thrombocyte-derived MVs, of T cell –derived Fas+ and FasL+ MVs, of trophoblast-derived HLA-G+ MVs and of mast cell- derived CD117 MVs. Our results could contribute to a more detailed understanding of the immunogenic effects transferred by MVs and of the processes leading to preeclampsia or preterm delivery often occurring in asthmatic women



P16

Histamine chloramine modifies adjuvant arthritis in rats

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We have previously shown that histamine chloramine (HisCl), endogenous compound formed at the inflammatory sites from HClO (activated neutrophils) and histamine (mast cells), inhibited *in vitro* reactive oxygen production in neutrophils via the H₄ receptor and modulated the course of experimental acute inflammation. In this study, we evaluated the effect of HisCl on adjuvant – induced arthritis (AA) in rats.

AA was induced in male Brown-Norway rats by injection of 0.1 ml of heat-killed *Mycobacterium tuberculosis* suspended in incomplete Freund's adjuvant into the foodpad. Rats were treated with HisCl (5ml 5Mm i.p.daily for 21 days. Pyrilamine maleate) (a histamine H₁ receptor antagonist) (10 mg/kg), cimetidine (a histamine H₂ receptor antagonist) (25 mg/kg), histamine receptor H₃ antagonist ciproxifan hydrogen maleate (0.14 mg/kg) and a histamine receptor H₃/H₄ antagonist thioperamide maleate) (2mg/kg) were administered i.p.daily for 21 days. Treatment was started simultaneously with AA.

Administration of HisCl during development of AA suppressed the generation of oxygen radicals in peripheral blood neutrophils. Cimetidine and thioperamide blocked the inhibitory effect of HisCl.

In the course of AA-induced inflammation elevated level of histamine in blood was found. HisCl caused additional increase in histamine level. This effect was blocked by H₂, H₃ and H₄ receptors antagonists.

This study shows that exogenous HisCl may modulate the course of AA through mechanism that partly involves stimulation of production/release of histamine and suppression of production of oxygen free radical species by blood neutrophils. HisCl exerts effects through its interaction with histamine H₂, H₃ and H₄ receptors.



P17

Histamine H₂ Receptor Expression in the Human Amnion Epithelium (HAE), Histamine-Evoked Interleukin(IL)-18 Secretion and Intensity of Pyroptosis in Chorioamnionitis (CHA).

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Increased apoptosis in HAE was reported in CHA. Previously, we suggested involvement of histamine and H₂ receptor-related pathway in caspase-1-dependent form of programmed cell death, called pyroptosis. Here, we examined comparatively (CHA vs normal pregnancy) the relationships between H₂ expression in HAE, IL-18 secretion in cultured amniotic epithelial cells (AEC) in response to histamine, and intensity of pyroptosis in corresponding cultures. Amnion samples were obtained after normal (Group II; N=12) and bacterial CHA-complicated pregnancies (Group I; N=12). Paraffin sections (5µm) were immunostained for H₂ receptors using a rabbit anti-human H₂ antibody. Quantitative immunohistochemistry was applied for evaluation of H₂ expression. Additionally, at the time of amnion sample collection, AEC were isolated using Okita's method and cultured *in vitro* for 12 days in normoxia. On Day 8, histamine (100µM), histamine + cimetidine (10µM), or histamine + anti-IL-18 antibody were added to the culture media in respective subgroups. Histamine-free controls were also analyzed. IL-18 level in the culture media was measured by ELISA every 3 days, beginning on Day 6. At Day 12, the cultures were terminated and quantitative determination of apoptosis/pyroptosis was performed using an immunoenzymatic assay (M30-Apoptosense ELISA Kit, Peviva AB, Sweden). Expression of H₂ in HAE was significantly ($p < 0.05$) increased in CHA. In histamine-free supernatants the differences in IL-18 levels were not significant, whereas histamine produced higher increase in IL-18 concentration in Group I, compared to Group II (4.8-fold vs 12.9-fold). Cimetidine strongly inhibited histamine-evoked increase in IL-18 levels. Apoptotic activity was higher in CHA, especially in the presence of histamine ($p < 0.02$). Both cimetidine and anti-IL-18 antibody inhibited histamine-related pyroptosis. In conclusion, overexpression of H₂ in HAE may determine stronger histaminergic activation of pyroptotic pathway in CHA.



P18

Phenotype and distribution of mast cells in cystic meningiomas

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Despite many reports concerning the association of mast cells with a variety of tumors, the functional significance of these cells in tumor biology remains obscure. The presence of mast cells has been interpreted as an immunological anti-tumor response or as a more direct interaction with tumor cells, especially interactions which facilitate tumor growth. Mast cells are a heterogeneous cell population and in human tissues heterogeneity of these cells is indicated by the different content of tryptase and chymase. Depending on the phenotype of mast cells, they provide different multifunctional cytokines and potent mediators such as histamine. Since the involvement of mast cells in the pathomechanism of the tumor cyst formation has been suggested, the aim of the present study was to determine the phenotype and distribution of mast cells in subtype of meningiomas with cystic changes.

Tumor specimens were obtained at craniotomy from patients undergoing tumor resection. Phenotypes and localization of mast cells in tissue sections were studied using mouse monoclonal antibodies directed against human tryptase and chymase. Biotinylated Ulex Europeans Agglutinin was used to study the distribution of capillary network in the tumor sections. Results show that some areas of tumors were completely devoid of vessels and those areas always contained cystic changes. All mast cells were tryptase immunopositive and one third of them contained chymase as well. It means that tryptase phenotype mast cells (MC-T) predominate over tryptase-chymase phenotype mast cells (MC-TC) in meningiomas with cystic changes. All mast cells were localized only in the vicinity of blood vessels and were numerous in these parts of tumors where the capillary network was well developed and dense. In the conclusion, we did not find a clear relationship of cystic changes in the tumor with the presence of mast cells but rather with the distribution of capillary network in the tumor specimens.



P19

Paradoxical effects of the EP₂ agonist, Butaprost, on histamine release from human mast cells

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Prostaglandin E₂ (PGE₂) inhibits IgE-dependent mediator release from human lung mast cells. Four PGE₂ receptors have been identified. Our studies indicate that PGE₂ acts through EP₂ receptors in mast cells (Kay *et al.*, 2006). This conclusion was based on the finding that the EP₂-selective agonist, butaprost, was effective at inhibiting mast cells. However, butaprost displayed some anomalous behaviour. The aim of this study was to explore the effects of butaprost on mast cells further.

Mast cells were generated by physical and enzymatic disruption of lung tissue. Mast cells were incubated (10 min) with antagonists/agonists before challenge with anti-IgE (25 min). In cross-desensitization experiments, mast cells were incubated (24 h) with PGE₂ or butaprost (10⁻⁵ M), the cells then washed after which the effects of the same agonists on IgE-mediated histamine release were investigated. Histamine released into supernatants was assayed using an automated fluorometric technique. In order to determine whether effects were statistically significant, ANOVA was performed.

PGE₂ and butaprost inhibited IgE-mediated histamine release from mast cells in a concentration dependent manner (n=7). Butaprost was less potent (logEC₅₀, 5.2±0.2) than PGE₂ (5.8±0.1) but more efficacious (E_{max} for inhibition: 87±4%) than PGE₂ (58±3%). The effects of the EP₂ antagonist, AH6809 (10⁻⁵ M), on the inhibition of histamine release by PGE₂ and butaprost were investigated. Whereas AH6809 (pK_B, 5.6±0.1) antagonized the effects of PGE₂, there was no effect on butaprost (n=5). In cross-desensitization experiments, incubation (24 h) of mast cells with PGE₂ or butaprost substantially attenuated (P<0.05) the subsequent ability of PGE₂ to inhibit histamine release but neither treatment (24 h) had any effect on the butaprost inhibition (n=4).

These data suggest that butaprost has effects on mast cells that are unrelated to interactions with EP₂ receptors.

Kay *et al.*, *Br J Pharmacol* 2006; 147:707-713



P20

Control of Human Basophil Activation by the SH2-Containing Inositol 5-phosphatase (SHIP)-1 is Dependent on the Nature of High-Affinity IgE Receptor Engagement.

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Human basophils rapidly generate and release important pro-inflammatory and immunomodulatory mediators upon high-affinity IgE-receptor crosslinking with allergens or anti-IgE. Here, we show that the ability of basophils to respond to IgE-mediated triggers is negatively associated with the level of constitutive SHIP-1 phosphorylation as well as the type of IgE-dependent stimulus employed. Basophils were obtained from buffy coats and highly purified by negative selection and magnetic cell sorting. Cells were incubated with a variety of IgE-dependent triggers together with unstimulated controls for varying periods in order to determine rate and magnitude of histamine release and intracellular signalling (by Western blotting). We observed that constitutive SHIP-1 phosphorylation (but not total SHIP-1 or Syk expressions) in unstimulated basophil preparations correlated closely with maximum basophil responses in terms of histamine release to anti-IgE. SHIP-1 was also associated with limiting basophil responses following supraoptimal concentrations of IgG-anti-IgE, a crosslinking agent that produced striking bell-shaped dose response curves. In contrast, there was no marked reduction in histamine release or increased SHIP-1 phosphorylation following supraoptimal stimulation of basophils with either concanavalin A or IgM-anti-IgE antibodies. The kinetics of basophil activation were also more rapid for the latter stimuli. These data show that overall basophil sensitivity to IgE-dependent activation depends on reduced constitutive SHIP-1 control but the pattern of dose-response curves generated by various IgE-mediated triggers differentially involves SHIP-1 input, depending on the properties of the crosslinking agent, such as IgE-binding affinity and valency.



P21

Isn't Histamine a unique amine?

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Oxidative deamination by monoamine oxidase enzymes (MAO A and B) plays an important role in amine neurotransmitter function and metabolism. While serotonin is exclusively metabolised by MAO A and N-*tele*-methylhistamine by MAO B, dopamine is a substrate of both MAOs in rat brain. Here, effects of *in vivo* ASS188 HCl, a mixed MAO A and B inhibitor, were examined and related to selective MAO A and B inhibitors, Clorgyline and Deprenyl, respectively. Wistar rats (n= 6-7 per group) were given subcutaneously, either ASS188 HCl (ASS188, 1mg/kg), clorgyline (Clorg, 0.3 mg/kg), deprenyl (Depr, 0.5mg/kg) or physiological saline (Contr, 1ml/kg) for 5 consecutive days. They were kept in metabolic cages, having free access to water and food. Consumption and excretion were daily recorded. Following decapitation of the rats 3-4 hr from the last injection, their brains were quickly removed. Dissected hypothalamus and remaining parts of the brain were immediately frozen in liquid nitrogen and stored until assayed. The substrates ¹⁴C labelled were used. Namely, serotonin and β -phenylethylamine for MAO A and B, respectively, spermidine for polyamine oxidase and 14C-methyl S-adenosyl-Lmethionine for histamine N-methyltransferase. 5HT, DA, NA and A concentrations in brain tissues were measured by RIA method with kits from DIAsource ImmunoAssays S.A., Nivelles, Belgium. Cerebral HA was determined by radioenzymatic assay and urinary HA was measured by fluorometry (360nm/450 nm), after column chromatography and derivatisation with o-phthaldialdehyde. The results show that ASS188-treated rats had only 5% of MAO A and 70% MAOB activity, expressed by their control pairs. ASS188 treatment exerted no effect, either on the brain PAO, HMT or on urinary histamine excretion. However, interestingly enough, in all the cerebral amines except histamine there was a significant increase in brain concentration. It was the most dramatic for dopamine (28-fold), lower for serotonin (8-12-fold) and noradrenaline (2-fold). For comparison, DA concentration was increased roughly 5-fold, following either Clorgyline or Deprenyl. Histamine concentration, was, on the contrary, decreased by over 30 percent in the hypothalamus and roughly 25 percent in the brain remnants, indicating an increased turnover rate. Neuronal histamine mobilisation is compatible with its postulated role as a dangerous response signal. Accordingly, released histamine promotes the activation of compensatory, homeostasis supporting mechanisms.

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P22

Comparison of the differentiation capacity of histamine free (HDC KO) and wild type cardiac stem cells

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Although the major functions of histamine (e.g. in allergic reactions or in cell proliferation) are known for several years, there is still little known about its role in cell differentiation.

As a continuation of our earlier work dealt with ES cell cardiac differentiation carried out in histamine free, HDC KO and wild type ES cells, now we tried to elucidate the role of histamine in the adult stem cell differentiation.

Cardiac stem cells were isolated from adult WT and HDC KO CD1 mice using a Millipore cardiac stem cell isolation kit. The expression of all those heart muscle specific markers - nestin, titin and desmin – which were tested on ES cells (results were presented on the 34th EHRS Symposium) were checked by real-time PCR in undifferentiated and differentiated cardiac stem cells, as well. In accordance with our observations made on ES cells when we noticed that HDC KO ES cell differentiation to cardiomyocytes took a longer time, cardiac stem cells of this genotype also showed a much slower cardiac differentiation rate. While nestin was expressed at a higher level in both stem cell types with the HDC KO genotype, desmin expression was higher in the WT ES cells, but not in the cardiac stem cells of the same genotype. However, titin which showed a high expression in the differentiating KO ES cells was not seen in the differentiating HDC KO cardiac stem cells at all. Id2 - as a cardiac conductance specific marker - expression was also tested and proved to be highly expressed in the histamine free cardiac stem cells. As for all checked immunocytochemical markers there were no differences between WT and HDC KO cardiac stem cells, however the intracellular distribution of Id2 was unusual.

Although these are preliminary results only, it further supports that histamine not only acts on heart beat at the time anaphylaxis or in the differentiating ES cells, but it also influences cardiomyogenesis from the early embryonic life till adulthood.



P23

Pharmacological Properties of UR-63325, a H₄R Antagonist in Clinical Development

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Histamine H₄ Receptor (H₄R) is mainly expressed in several lymphopoietic cells in humans and rodents and has been proposed to be an interesting target for immunoinflammatory pathologies [1]. Our objective was the pharmacological characterization of UR-63325, a new H₄R antagonist discovered and developed at Palau Pharma S.A. UR-63325 showed high affinity for human (K_i = 15 nM), mouse, rat, and monkey H₄ receptors (x0.56, x1.04 and x098 fold vs. human, respectively). Saturation assays with the tritiated compound showed fast association and dissociation properties from the human receptor and K_d = 11 nM. It was very selective vs. the other histamine receptors (>150 fold), as well as vs. a wide panel of GPCRs, nuclear receptors, enzymes and bioamine transporters. In eosinophils, UR-63325 behaves as antagonist as it potently reduced histamine-induced shape change, both in isolated cells (IC₅₀ = 10 nM) and in whole-blood conditions (IC₅₀ = 47 nM). Moreover, it reduced histamine-induced eosinophil chemotaxis at 1 μM. This compound has been evaluated in the zymosan-induced peritonitis model in mouse and significantly reduced cellular infiltration in the peritoneum at 50, 30 and 10 mg/kg. UR-63325 shows a very interesting *in vitro* profile, as well as a promising *in vivo* activity and has recently entered in Phase I clinical trials for asthma and allergic rhinitis.

[1] Zampeli E, Tiligada E. Br J Pharmacol 2009; 157, 24–33



P24

Synthesis and Structure-Activity Relationships of Conformationally Constrained Cyanoguanidines:

Potent and Selective Histamine H₄ Receptor Agonists

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Results of different investigations suggest the hH₄R to play a crucial role in immunological and inflammatory processes [1]. To further explore the (patho)physiological function of the hH₄R selective ligands – both agonists and antagonists – are needed.

Very recently, we identified imidazolylalkylcyanoguanidines as highly potent and selective hH₄R agonists [2]. Highest potency resided in compounds with a tetramethylene linker, connecting the imidazole and the cyanoguanidine moiety as in UR-PI376. Since another cyanoguanidine, the conformationally constrained tetrahydrofurane derivative OUP-16 [3] was reported to have a 40-fold selectivity for the H₄R over the H₃R, and substances with reduced flexibility might help to suggest or improve H₄R-ligand interaction models, we synthesized a small series of compounds with a cyclopentan-1,3-diyl linker, thereby combining the structural features of UR-PI376 and OUP-16.

Potencies, intrinsic activities and selectivities of these compounds were determined in GTPγS assays using membrane preparations of Sf9 insect cells, expressing the respective hHR subtype. After enantiomeric separation of the most promising racemates with the help of HPLC on a chiral stationary phase, the most potent and selective hH₄R-agonist was identified as *trans*-(+)-UR-RG98 (EC₅₀ = 11 nM, E_{max} = 0.75), displaying a more than 100-fold selectivity over the hH₃R (weak antagonism). Surprisingly, the corresponding *trans*-(-) enantiomer showed no hH₄R agonistic activity at all, by contrast, the two *cis*-isomers are moderately potent hH₄R agonists (EC₅₀ ~ 250 nM).

[1] de Esch, I.; et al.; *Trends Pharmacol. Sci.* **2005**, 26, 462–469.

[2] Igel, P., et al.; *J. Med. Chem.* **2009**, 52, 6297-6313.

[3] Jablonowski, J. A.; et. al *J. Med. Chem.* **2003**, 46, 3957-3960.



P25

An *In Silico* Study of Interactions Between Mammalian Histidine Decarboxylase and its Inhibitor Epigallocatechin 3-Gallate

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Background: Epigallocatechin 3-gallate (EGCG) is an antioxidant, antitumoral and antiangiogenic compound present in green tea. Our group has described inhibitory effects of EGCG on inflammatory cells, and a potent inhibition of mammalian histidine decarboxylase (HDC), the enzyme responsible for histamine synthesis. However, there is no insight concerning the actual interactions between both molecules.

Objectives: Our aim is to use molecular docking simulations to study HDC-EGCG interactions.

Methods: A number of biocomputational tools were used in this study, including the programs H++, ADT, AutoGrid 4, AutoDock 4, MacPyMol, TextWrangler and GRAMM.

Results: Since there was no clear evidence of the place of interaction of EGCG with the active, dimeric HDC, we started with a “blind screening” over the dimerization surface of a monomeric HDC. The analysis of the docking simulations revealed three putative interaction zones. In the first one, the conformation of EGCG would allow it to contact amino acid residues important for catalysis. In the second one, EGCG could interact with the so-called flexible loop of HDC, which suffers an important conformational change during enzyme catalysis. In the third zone, EGCG cannot interact with important residues for catalysis; however, due to its favourable union energy, an interaction of EGCG with mature monomeric HDC before dimerization cannot be ruled out.

Conclusions: Three zones for putative HDC-EGCG interactions have been found. Further studies should elucidate which of these interactions has the greatest possibility to occur under physiological conditions.

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P26

Dual blockers of histamine H₃ receptors and norepinephrine transporter for the treatment of pain

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Norepinephrine (NE) has a well recognized role in control of pain, and it has been suggested that H₃ receptor antagonists produce their analgesic effects in preclinical models of nociception through modulation of NE release. We hypothesized that the combination of selective NE transporter (NET) blockade with H₃ antagonism in one molecule could have enhanced utility over the single mechanisms, with either increased efficacy or improved tolerability. Two structurally distinct compounds were developed with high potency (H₃ and NET K_i = 5-40 nM), CNS penetration, and good rat PK. A dual H₃-NET inhibitor has much improved GI tolerability compared to duloxetine. All three are fully efficacious and potent against MIA-induced knee joint pain with ED₅₀ 0.3-2 mg/kg. However, probing the scope of efficacy in other pain models found the dual blocker is ineffective. The overall conclusion is that although the dual blocker compounds have good efficacy and potency in the MIA model, they fail to improve the magnitude and breadth of analgesic efficacy compared to single-mechanism agents.



P27

Expression profiling of chemotactic receptors in inflammatory diseases

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Inflammatory diseases, such as Inflammatory Bowel Disease (IBD), Chronic Obstructive Pulmonary Disease (COPD) and rheumatoid arthritis, are associated with a significant accumulation of leukocytes in the respective tissues. Chemokines and their receptors are key mediators involved in the infiltration of leukocytes. Chemokine receptors belong to the class of G protein coupled receptors (GPCRs) and are therefore considered important drug targets for treatment of inflammatory diseases. In addition, also other chemotactic GPCRs, like the histamine H₄ and C5aR1, were found to be expressed on subsets of leukocytes. We have established a quantitative PCR array to define the expression levels of the different chemotactic receptors in patient (e.g. IBD) and control material. Validation of expression of the chemotactic receptors is performed by Western blot analysis, immunohistochemistry and/or binding studies. For example, we have shown H₄ expression with immunohistochemistry on both inflamed and non-inflamed tissue of IBD patients. This approach is a first step to define novel targets in inflammatory diseases.



THE INTERNATIONAL ANTHEM OF
THE EUROPEAN HISTAMINE RESEARCH SOCIETY

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

1. We talk of toxicosis / migraine, shock or halitosis
Singing Histaminosis all the day.
Trauma, burns and inflammation / headache, pain and constipation,
Singing Histaminosis all the day.
2. You give asthmatic wheezes / the allergic sneezes,
Singing Histaminosis all the day.
Though obscure as yet, the fact is / you're involved in anaphylaxis,
Singing Histaminosis all the day
3. Since the time of Dale and Barger / your files are longer, larger
Singing Histaminosis all the day.
The control of circulation / then gastric stimulation,
Singing Histaminosis all the day.



CHORUS

4. Mast cells by the dozen / and basophils, your cousin,
Singing Histaminosis all the day.

They come and they go / fluctuate to and fro,

Singing Histaminosis all the day

5. We heard a lot of groaning / from the upstart, Serotonin,
Singing Histaminosis all the day.

Down with 5-hydroxytrypta / and up with good old hista,

Singing Histaminosis all the day

6. Each year we meet in May / to concentrate and play,
Singing Histaminosis all the day

What luck to have such friends / to cater for our trends,

Singing Histaminosis all the day

CHORUS



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

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

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

CHORUS

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

11. Then to Budapest we went / with Susan on the scent,
Singing histaminosis all the day.



West Germany again / for Hannover by name,

Singing Histaminosis all the day

12. In nineteen eighty two / to Bled we all flew,

Singing Histaminosis all the day.

Then Brighton to the fore / with sea breezes by the shore,

Singing Histaminosis all the day.

CHORUS

13. And in nineteen eighty four / back in Florence like before,

Singing Histaminosis all the day.

Then in Aachen eighty five / Charlemagne became alive,

Singing Histaminosis all the day.

14. Then in Odense in Spring / in the Castle we did sing,

Singing Histaminosis all the day.

And then Czecho was the next / with our Rado at his best,

Singing Histaminosis all the day.

15. G.B. West was then cheered / for the ten years we'd been steered,

Singing Histaminosis all the day.

To Copenhagen again / we're invited there by Svend

in the year eighty eight in lovely May.



CHORUS

16. And in nineteen eighty nine / it was also very fine,
we're in Holland for the very first time.

To Kuopio in Finland / to the beautiful, but cold land,
we were watching the Finnish chopping wood.

17. Then to Marburg we returned / ninety one and also learned
that histamine in surgery is bad.

The next year we met again / Manuel in sunny Spain,
Singing ai, ai and olé all the way.

18. Then with Eddy on the Rhine, we had more beer than wine,
Singing histaminosis all the day.

To Zsuzsanna ninety four / we went back to Danube shore,
Singing Histaminosis all the day.

CHORUS

19. Then with Igor ninety five / and the Volga was alive
And we entered the Russian Golden Ring.

In Antwerpen ninety six / Frans did show us a few tricks,



Singing Histaminosis all the day.

20. To Sevilla, once again / we all met in lovely Spain,

Singing Histaminosis all the day.

To Agnieszka ninety eight / back in Poland it was great,

Singing Histaminosis all the day.

21. Then to Lyon ninety nine / and Histamine's still mine

Singing Histaminosis all the day.

New Millenium in Rome / Bruno made us all feel home

Singing Histaminosis all the day.

CHORUS

22. Pertti took us on a boat / we and Histamine could float

So to Turku we came two thousand one.

Andras called two thousand two / and to Eger did we go

A Hungarian meeting once again

23. In the year two thousand three / we could lots of tulips see

Now Henk Timmerman was host in Amsterdam

Back to Germany next spring / and with Helmut did we sing

Singing Histaminosis all the day



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

CHORUS

25. Returned to Florence the next year / For the third time we were here
And for us Emanuela made the day!
Back to Stockholm that we knew / with a lovely water view
With Anita in the North two thousand eight.
26. Then to Fulda the next year / we're in Germany to hear
How our Frido with Histamine can play.
To **Durham** we went then / in the year two thousand ten.
And with **Paul** near Cathedral did we stay.

CHORUS



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