41st Annual Meeting of the EHRS, held jointly with COST Action BM0806 Belfast, May 2-5 2012

Programme and Abstract Book

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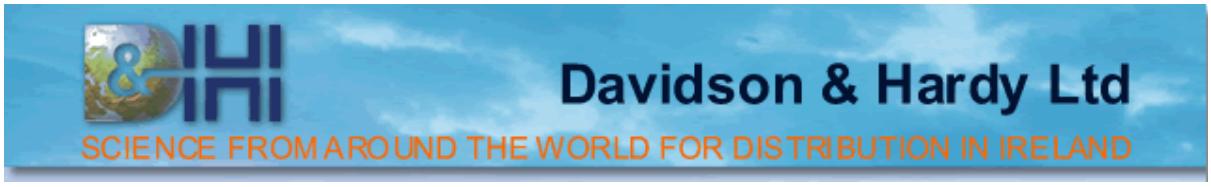
Sponsors

Conference



Sponsors continued

Young Investigator's Award

EHRS

Student Travel Bursaries

EHRS (n=6)
ESK Assem Student Bursary (n=2)
GB West Memorial Trust

Previous EHRS Meetings

1970's		1980's		1990's		2000's		2010's	
		1980	Visegrad	1990	Kuopio	2000	Nemi(Rome)	2010	Durham
1971	Lodz	1981	Hannover	1991	Marburg	2001	Turku	2011	Sochi
1972	Paris	1982	Bled	1992	Malaga	2002	Eger		
1973	Marburg	1983	Brighton	1993	Cologne	2003	Noordwijkerhout		
1974	Copenhagen	1984	Florence	1994	Budapest	2004	Bergisch-Gladbach		
1975	Florence	1985	Aachen	1995	Moscow	2005	Bled		
1976	Paris	1986	Odense	1996	Antwerp	2006	Delphi		
1977	London	1987	Strbske Pleso	1997	Seville	2007	Florence		
1978	Lodz	1988	Copenhagen	1998	Lodz	2008	Stockholm		
1979	Stockholm	1989	Breda	1999	Lyon	2009	Fulda		

Welcome

Dear Friends

On behalf of the Organizing Committee, I would like to welcome you to Belfast for the 41st meeting of the European Histamine Research Society. The meeting will start with the Welcome Party on 2nd May and end with breakfast on 6th May. I am certain that between these times, we will all learn a lot of new information about histamine and its receptors, will have established some new scientific collaborations and had time to meet up with old and new friends.

This is the first time that the EHRS has come to the island of Ireland and we are very happy to welcome you here. The congress venue is the Hilton Hotel in the centre of Belfast. We have negotiated excellent rates to stay there, so I hope that you will take advantage of this. There will, of course, be a partner programme for those not attending for the science.

Belfast is at the far west of Europe – a long way from our last meeting in wonderful Sochi! People have lived in the Belfast area since the Bronze Age and there is a henge at the Giants Ring near Shaws Bridge. However, Belfast really started to develop in the 17th century. The name Belfast comes from the Gaelic Beal Feirste or mouth of the sandy ford. Belfast was the most important industrial town and later city in the island of Ireland. Belfast was famous for its linen and rope making, its tobacco and of course its ship building and more recently aircraft building. Many of these industries have declined but Belfast is now a vibrant city with excellent restaurants and pubs, a thriving cultural scene and great shopping!

The scenery is stunning. If you can spend some extra time here, you will love it. We have beautiful bays, white sand, great hills (though we call them mountains!), palm trees! On our outing we will go to the North Antrim coast to see the UNESCO World heritage site – the Giant's Causeway. This was built by the giant Finn MacCool or, alternatively, due to igneous activity in the early Tertiary period (62-65 million years ago). Personally, I think that the stones look like benzene rings – but you will have to wait and see for yourself!!

The weather in May can be a bit variable – the average low is 6 °C and the average high is 16 °C, with a good chance of some rain!! So I suggest you bring a light waterproof jacket among your other items!!

Travel connections to Belfast are excellent – we have 2 airports: The George Best Belfast City Airport (3 miles from the city centre) and the Belfast International Airport (17 miles away). In addition, many cheap flights go to Dublin (100 miles away, ca. 20€ return bus fare, 2 h 30 min).

Welcome to Belfast! I look forward to seeing you in my adopted home.

Best wishes

Madeleine Ennis
Organizer

Organizing Committee

Madeleine Ennis - Chair
Karim Dib
Fred Pearce
Astrid Sasse
Anita Sydbom
Gill Sturman
Dawn Wylie
Stuart Haines (photographer)

Abstract Evaluation Committee

Anita Sydbom
Frank Ahrens
Pierre Chatelain
Marlon Cowart
Pertti Panula
Elena Rivera
Hubert Schwelberger
Gill Sturman

Bursary Committee

Anita Sydbom
Frank Ahrens
Pierre Chatelain
Marlon Cowart
Pertti Panula
Elena Rivera
Hubert Schwelberger
Gill Sturman

COST ESR Awards Committee

Madeleine Ennis
Katherine Tiligada

Young Investigator Award Committee

Pertti Panula - Chair
Marlon Cowart
Bernie Gibbs

Poster Prize Committee

Paul Chazot - Chair
Kim Barrett
Hubert Schwelberger
Jerzy Jochem

General Information

Host Institute
Centre for Infection and Immunity, Health Sciences Building, School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, 97 Lisburn Road, Belfast, BT9 7BL
Telephone: 0044 2890 972672
Email: EHRS2012@qub.ac.uk OR m.ennis@qub.ac.uk

Congress Venue

Hilton Belfast hotel
4 Lanyon Place Belfast BT1 3LP, United Kingdom
Telephone: 0044 289 027 7000

Registration Desk
The registration desk at the mezzanine level will be open as follows

Wednesday	2nd May	15.00 -20.00
Thursday	3rd May	08.00 -12.00
Friday	4th May	08.00 -12.00
Saturday	5th May	08.00 -12.00

Conference Website
http://www.qub.ac.uk/EHRS2012

Oral Communications
Oral presentations should last no longer than 10 min and there will be 5 min for discussion. The presentations should be in MS Power Point format. A PC equipped with MS PowerPoint for Data Projection will be available in the Conference Venue. The projector is only PC and NOT Mac compatible, except for Mac software after 2005. The presentation should be brought on a USB pen drive, saved in a file as: Name_of_presenter.ppt. Presentations should be sent to the organizer by Friday April 27th (EHRS2012@qub.ac.uk).

Poster Presentations
Authors should present their posters during the assigned session. The main point(s) from the poster will have to be presented in 2 min, followed by a 3 min discussion. The posters will be on display throughout the meeting. Presenters are to stand by their posters during the coffee breaks and the poster session.
The maximum poster size is 120 x 90 cm (h x w).
Material to attach the posters will be provided. Posters which have made the final short-listing for the poster prizes will be revisited by the Poster Jury on Saturday afternoon. Prizes will be announced at the Farewell Dinner.

Social Events

Welcome reception (Wednesday 2nd May) from 20.00 onwards in the Hilton Hotel (Lagan Suite).

On our outing (Thursday 3rd May), we will visit the Norman Castle in Carrickfergus and then drive along the **North Antrim coast** to see the UNESCO World heritage site – the **Giant's Causeway**. This was built by the giant Finn MacCool or, alternatively, due to igneous activity in the early Tertiary period (62-65 million years ago). You need rain jackets (it's Ireland!) and comfortable shoes – stiletto heels are not advised! We will have dinner in the beautiful **Galgorm Manor**, where we will be entertained by some traditional Irish music from "Pure Blarney". See: http://www.pureblarney.com/

On Friday 4th May, after dinner in the Lagan Suite, we will indulge in some Irish Whiskey tasting!

On Saturday 5th May, after dinner in the Sonoma Restaurant in the Hilton, we will have a bit of a Ceilidh with 'Haste to the Wedding'.
See: http://www.hastetothewedding.com/index.htm

Partner Programme

Thursday morning attend Opening Session and Honorary Membership Ceremony, then at leisure or at conference till the Outing starting at 12.30.

Friday

Meet by the registration desk at 9.30 then Bus Tour of Belfast, lunch at Hilton with delegates and then meet by the registration desk at 14.00 and visit the Titanic Exhibition Belfast.

Saturday

Meet by the registration desk at 9.30 then trip to Lisburn to visit The Irish Linen Centre and Lisburn Museum; lunch at the Hilden Brewery Lisburn and then on to Ardress House.

The Programme at a Glance

Wednesday May 2nd 2012

15.00-20.00	Registration and poster mounting at the Belfast Hilton Hotel
16.00-18.30	EHRS Council Meeting, Tower Suite
19.00-20.00	Meeting of WG1 COST BM0806, Board Room
20.00 – onwards	Welcome reception, Lagan Suite

Thursday May 3rd 2012

8.00 -12.00	Registration and signing of COST attendance register
8.00 onwards	Poster Mounting
8.30 – 9.00	Opening Session
9.00-9.45	Honorary Membership Ceremony
9.45-10.30	Professor Holger Stark (Frankfurt University, Germany) "Histamine H_4 receptor – ligand, binding and activation." – COST BM0806
10.30-11.00	Coffee break and poster viewing
11.00- 12.15	Walter Schunack Memorial Symposium
12.30 onwards	Outing

Friday May 4th 2012

8.30 – 12.00	Registration and signing of COST attendance register
8.30-9.15	G.B. West Lecture – Professor Peter Bradding (Leicester University, UK) "Interactions between mast cells and structural airway cells in the pathogenesis of asthma"
9.15-11.20	Coffee and poster session
11.20-13.05	Novel highlights on mast cell functions - COST BM1007
13.05-14.15	Lunch and meeting of WG2 COST BM0806 (Board Room)
14.15-15.15	Round Table – What's new in H_4 research? - COST Action BM0806
15.15-16.00	Coffee & Poster Session
16.00-17.00	Oral Session
17.00-18.00	Oral Session
18.00-19.15	Meeting of WG3 COST BM0806 (Board Room)

20.00 Dinner (Lagan Suite)

Saturday May 5rd 2012

8.30 -12.00	Registration and signing of COST attendance register
8.30-9.15	Professor M. Beatrice Passani (University of Florence, Italy) "H_4R and neuroinflammation: insights from mouse experimental autoimmune encephalomyelitis" – COST Action BM0806
9.15-10.30	Oral Session
10.30-11.45	Coffee & Poster session
11.45-13.00	Oral Session
13.00-14.00	Lunch and meeting of WG4
14.00-15.15	Young Investigator Award Oral Session to include COST Action BM0806 ESR/STMS presentations
15.15-15.45	Coffee & Poster viewing of the selected posters
15.45-16.00	Dr Katherine Tiligada (University of Athens, Greece) "Progress and limitations in H_4R research" – COST Action BM0806
16.00-17.45	General Assembly of the EHRS
18.00-19.30	Meeting of the MC COST Action BM0806 (Board Room)
20.00	Farewell Dinner (Sonoma Restaurant, Hilton Hotel)

Scientific Programme

Wednesday May 2nd 2012

15.00-20.00	Registration at the Belfast Hilton Hotel
18.00-20.00	Poster mounting
16.00-18.30	EHRS Council Meeting
19.00-20.00	Meeting of WG1 COST Action BM0806
20.00 – onwards	Welcome Reception

Thursday May 3rd 2012

8.00 -12.00	Registration and signing of COST attendance register
8.00 onwards	Poster Mounting
8.30 – 9.00	Opening Session
9.00-9.45	Honorary Membership Ceremony
9.45-10.30	**L1 "**Histamine receptor – Ligand, Binding and Activation." Professor Holger Stark (Frankfurt University, Germany) - COST Action BM0806 *Introduced by Astrid Sasse*
10.30-11.00	Coffee & Poster Session
11.00- 12.15	**Walter Schunack Memorial Symposium** *Chaired by Holger Stark and Stephany Micallef (ESR)*
11.00-11.15	"Professor Walter Schunack – an appreciation." Holger Stark
11.15-11.30	**O1** Ligand-directed H_4R signalling: a step forward to optimized H_4R drugs? *S. Nijmeijer, E.M. Rosethorne, H.F. Vischer, S.J. Charlton, R. Leurs*
11.30-11.45	**O2** Influence of the lipophic part of 3-(1*H*-Imidazol-4-yl)propyl carbamates on histamine H_3/H_4 receptor affinity, selection and potency. *M. Więcek, T. Kottke, R. Seifert, H. Stark, K. Kieć-Kononowicz*
11.45-12.00	**O3** Differential binding kinetics of histamine H_4 receptor ligands: from quick leavers to long-stay binders. *H.D. Lim, S. Nijmeijer, H.F. Vischer, C. de Graaf, R.A. Smits, I.J.P. de Esch, R. Leurs*

12.00-12.15 **O4** Histamine H_3 receptor regulates the functions of pancreatic β-cell.
T. Nakamura, T. Yoshikawa, N. Noguchi, F. Naganuma, R. Harada, A. Mohsen, K. Yanai

12.30 onwards Outing

Friday May 4th 2012

8.30 – 12.00 Registration and signing of COST attendance register

8.30-9.15 **L2** **G.B. West Lecture** - "Interactions between mast cells and structural airway cells in the pathogenesis of asthma."

Professor Peter Bradding (Leicester University, UK)

Introduced by Pete Peachell

9.15-11.20 **Coffee & Poster Sessions 1 and 2**

Poster Session 1 **Histamine and the nervous system**
Chaired by Paul Chazot and Arianna Carolina Rosa

P1 Understanding how histamine induces neuron differentiation.
G. Rodríguez, I. Velasco, G. García-López, H. Flores-Herrera, N.F.Díaz, A. Molina-Hernández

P2 Behavioural analysis of H_4 receptor knockout mice.
K. Rossbach , M. Bankstahl,W. Bäumer

P3 Discovery of novel H_1R and H_3R modulators through a multiplexed screening strategy utilizing small molecules from a CNS DOS focused library
E. Holson, J.T. Lowe, S. Kesavan, L.A. Marcaurelle, J. Beaudouin, E. Comer, E. Davoine, K. Dennehy, J.R. Duvall, T. Hanania, M.D. Lee, IV, M. Lewis, D. Lowe, J.-C. Marie, C.A. Mulrooney, G. Muncipinto, T. Petryshen, B-C. Suh, F. Wagner, J. Wei, M.A. Foley

P4 H_1R signalling in antigen presenting cells is dispensable for eliciting pathogenic T cells in experimental allergic encephalomyelitis.
N. Saligrama, R. Noubade, L. K. Case, M. E. Poynter, C. Teuscher

P5 Inhibition of depolarization-evoked [3H]-dopamine release by the activation of the human histamine H_3 receptors of 445 and 365 amino acids expressed in human neuroblastoma SH-SY5Y cells.
G. Nieto-Alamilla, J. Escamilla-Sánchez, R. González-Pantoja, J.-A. Arias-Montaño

P6 The histamine H_3 receptor antagonist A-960656 is effective in animal models of osteoarthritis and neuropathic pain.
G.C. Hsieh, M.D. Cowart, L. Black, C. Zhan, E. J. Gomez, M. Pai, M.I. Strakhova, A.M. Manelli, T.L. Carr, T.R. Garrison, J. D. Brioni

P7 The novel histamine H_3 receptor antagonist ST-1283 attenuates ethanol consumption and preference in mice.
B. Sadek, A. Bahi, M. Walter, J. S. Schwed, T. Kottke, H.Stark

P8 Histamine promotes neuronal differentiation of cultured midbrain neural precursors, but diminishes dopamine neuron numbers in vitro and in vivo.
I. Escobedo, A. Molina-Hernández,I. Velasco

P9 Involvement of the central histaminergic system in thyrotropin releasing hormone-induced resuscitating effect in haemorrhagic shock in rats.
J. Jochem

Poster Session 2 Mast cells, metabolism and chemistry
Chaired by Kim Barrett and Kristine Rossbach

P10 Intravenous infusion of ascorbic acid reduces plasma histamine levels.
A.F. Hagel, C.M. Layritz, W.H. Hagel, H.J. Hagel, E. Hagel, A. Rosenberg, W. Dauth, J. Kressel, M.F. Neurath, M. Raithel

P11 Exogenous salsolinol acts on mast cells and interstitial cells of Cajal in the rat.
M. Kurnik, K. Gil, A. Bugajski, P. Thor

P12 Ultra high performance liquid chromatography for histamine and methylhistamine in tissues and plasma.
S. Rajtar Osredkar

P13 Diamine oxidase is present in seminal plasma from man but not in that from other mammals.
F. Ahrens, J. Feurle, H.G. Schwelberger

P14 Characterization of the EP receptor expressed by human lung mast cells using novel EP receptor antagonists.
L.J. Kay, M. Gilbert, S. Skerratt, N. Pullen, P.T. Peachell

P15 Effect of PKC on HMC-1^{560} and HMC-$1^{560,816}$ mast cell lines activation.
A.Tobío, A. Alfonso, L. M. Botana

P16 Novel mast cell-stabilising amine derivatives of 3,4 dihydronaphtholen-1(2H)-one and 6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one.
J.W. Barlow, T. Zhang, O. Woods, J.J. Walsh

P17 High affinity dimeric H_3R ligands indicate receptor dimers/oligomers.
K. Wingen, S. Schwed, L. Weizel, E. Proschak, H. Stark

P18 Interaction of N-methyl piperazine derivatives with PTEN promoter.
P. Kechagioglou, R. Papi, G. Latacz, D.A. Kyriakidis, K. Kieć-Kononowic

11.20-13.05 Novel highlights on mast cell functions - *COST BM1007*
Chaired by Ulrich Blank and Francesca Levi- Schaffer

11.20-11.50 **L3** "Late signaling events for histamine release by mast cells."
Ulrich Blank (INSERM, Paris, France)

11.50-12.20 **L4** "Eicosanoids - performing lipidiomics on activated mast cells."
Gunnar P. Nilsson (Karolinska Institutet, Solna, Sweden)

12.20-12.50 **L5** "The allergic effector unit: mast cells - eosinophils interactions regulate the allergic response."
Francesca Levi-Schaffer (The Hebrew University of Jerusalem, Jerusalem, Israel)

12.50-13.05 **O9** Histamine downregulates skin barrier proteins – a potential role in the pathogenesis of atopic dermatitis
M. Gschwandtner, M. Mildner, V. Mlitz, L. Eckhart, T. Werfel, R. Gutzmer, E. Tschachler

13.05-14.15 Lunch and meeting of WG2 COST Action BM0806

14.15-15.15 Round Table – What's new in H_4 research? - COST BM0806
Organized by Rob Thurmond.
Participants are:-
Elena Rivera, Paul Chazot, Ralph Gutzmer and Holger Stark

15.15-16.00 Coffee and poster viewing

16.00-17.00 Mast cells and Inflammation
Chaired by Wilfried Lorenz and Linda Kay

16.00-16.15 **O5** Differential Role of Hypoxia-Inducible Factor-1 in Human Mast Cell and Basophil Responses.
B.F. Gibbs, I. Yasinska, V.V. Sumbayev

16.15.-16.30 **O6** Desensitization of IgE-dependent responses in human lung mast cells following exposure to anti-IgE or antigen.
A. Lewis, P.T. Peachell

16.30-16.45 **O7** Allergic Airway inflammation in H_2R knockout mice: increased susceptibility independently of Treg cell numbers.
R. Ferstl, P. Konieczna, M. Ziegler, R. Frei, C.A. Akdis, L. O`Mahony

16.45-17.00 **O8** Histamine contributes to human inflammatory joint disease by increasing the rank/OPG ratio through altered NR4A activity in chondrocyte cells.
A. Gilmore , V. Marzaioli, H. Angerer, J.P. McMorrow, D. Zocco, A.N. McEvoy, M.H. Stradner, E.P. Murphy.

17.00-17.45 **Clinical aspects of the H_4 receptor** - COST BM0806
Chaired by Jorge Brioni and Liam O'Mahony

17.00-17.15 **O10** Blood histamine levels and skin H_4R expression in patients with chronic spontaneous urticaria.
M.P. Makris, N.L. Lethbridge, E. Zampeli, X.S. Aggelides, P.L. Chazot, E. Tiligada

17.15-17.30 **O11** Antagonism of the histamine H_4 receptor reduces LPS-induced TNF production in vivo.
J.M. Cowden, F. Yu, M. Challapalli, J.-F. Huang, W.-P. Fung-Leung, J.Y. Ma, J.P. Riley, M. Zhang, P.J. Dunford, R. L. Thurmond

17.30-17.45 **O12** Therapeutic potential of H_4R agonists in an experimental model of human breast cancer.
D. Martinel Lamas, M. Croci, L. Sambuco, E. Carabajal, N. Massari, R.M. Bergoc, E.S. Rivera, V.A. Medina

18.15-19.15 Meeting of WG3 COST Action BM0806

20.00 Dinner

Saturday May 5th 2012

8.30 -12.00 Registration and signing of COST attendance register

8.30-9.15 **L6** **"**H_4R and neuroinflammation: insights from mouse experimental autoimmune encephalomyelitis."

Professor M. Beatrice Passani (University of Florence, Italy) – COST Action BM0806

Introduced by Emanuela Masini

9.15-10.30 Histamine and the nervous system

Chaired by Helmut Haas and Anayansi Molina-Hernández

9.15-9.30 **O13** Waking action of ursodeoxycholic acid depends on histamine and $GABA_A$ receptor block.
H.L. Haas, Y. Yanovsky, S.R. Schubring, Q. Yao, Y. Zhao, S. Li, A. May, J.S. Lin, O.A. Sergeeva

9.30-9.45 **O14** Lack of effects by ABT239, a histamine H_3 receptor antagonist, in histamine-deficient animals.
P. Blandina, G. Provensi, L. Munari, M.G. Giovannini, T. Garrison, J.R. Koenig, M. Cowart, J. Brioni, M.B. Passani

9.45-10.00 **O15** Combinatorial roles for histamine H_1-H_2 and H_3 -H_4 receptors in autoimmune inflammatory disease of the central nervous. system.
N. Saligrama, R. Noubade, L. K. Case, R. Del Rio, C.Teuscher

10.00-10.15 **O16** Human astrocytes transport histamine through plasma membrane monoamine transporter.
T. Yoshikawa, F. Naganuma, T. Nakamura, T. Iida, R. Harada, A. Mohsen, K. Yanai

10.15-10.30 **O17** Dynamic regulation of histaminergic and dopaminergic networks in zebrafish brain.
P. Panula, M. Sundvik , S. Semenova, Y.-C. Chen

10.30-11.45 Coffee and Poster Sessions 3 and 4

Poster session 3 Histamine – H_4 receptor

Chaired by Hubert Schwelberger and Saskia Nijmeijer

P19 The histamine H_4 receptor is overexpressed in the kidney of diabetic rats.

A.C. Rosa, C. Grange, M.A Katebe, E. Benetti, M. Collino, G. Miglio, G. Camussi, P.L. Chazot, R. Fantozzi

P20 H_1 versus H_4 anti-histamines in human neutrophil oxidative burst.
R. Nosal, K. Drabikova, V. Jancinova, T. Perecko, A. Lojek, M. Ciz, J. Kralova

P21 Effects of histamine H_4 receptor agonists and antagonists on carrageenan-induced inflammation in rats.
M. Adami, G.Coruzzi, R.Smits, H.Lim, R.Leurs

P22 Combination of prototypical histamine H_3 and H_4 receptor pharmacophores.
A. Schreeb, S. Schwed, L. Weizel, H. Stark

P23 Derivatives of 2-amino-1,3,5-triazine as new histamine H_4 receptor ligands.
K. Kamińska, M. Więcek, T. Kottke, S. Schwed, R. Seifert, H. Stark, J. Handzlik, K. Kieć-Kononowicz

P24 Histamine and Clozapine treatments inhibit tumour growth and increase median survival in human melanoma xenograft model.
N. Massari, V.A. Medina, M. Croci, L. Sambuco, D. Martinel Lamas, R.M. Bergoc, E.S. Rivera

P25 H_3 and $H_{3/4}$ antagonists modulate cytokine synthesis in patients with acute sensorineural hearing loss.
R.Khanferyan, L.Lazareva **WITHDRAWN**

P26 Comparative mRNA expression profiling in non-stimulated versus H_4R-stimulated human lymphocytes.
S.Mommert, D.Pischke, O.Dittrich-Breiholz, M.Kracht, H.Stark, R.Gutzmer, T.Werfel

P27 Specific histamine H_4 receptor antagonists act as potent modulators of the mammalian vestibular function.
E. Wersinger, G. Desmadryl, S. Gaboyard-Niay, A. Brugeaud, J. Dyhrfjeld-Johnsen, C.Chabbert

P28 Differential changes in H_4R expression in acute and chronic inflammatory pain models.
M. Katebe, N. Lethbridge, P.L. Chazot

P29 Effects of 2,4-diaminopyrimidine H_4R ligands on the histamine levels in the normal and inflamed rat conjunctiva.
S. Chalkiadakis, M. Walter, H. Stark, E. Tiligada

P30 Microsatellite repeat expansions in the human histamine 4 receptor gene.
S. Micallef, E. Quinn, D. Morris, A.Sasse

Poster session 4 **Clinical aspects and cellular studies**
Chaired by Jerzy Jochem and Karim Dib

P31 Histamine modulates salivary secretion and diminishes the progression of periodontal disease in rat experimental periodonititis.
J.P. Prestifilippo, E. Carabajal, M. Croci, J. Fernández-Solari, E.S. Rivera, J.C. Elverdin, V.A. Medina

P32 Histamine in paroxysmal atrial fibrillation (AF): results from an unselected population at a Tertiary University Emergency Unit.
M. Raithel, C.M. Layritz, A.F. Hagel, M.F. Neurath, W.G. Daniel, D. Ropers

P33 The effects of histamine and 4-methylhistamine on the oxidative burst of human leukocytes.
O. Vasicek, A. Lojek, M. Ciz

P34 Mast cells, endothelial proliferation and capillary tube formation in myocardia of patients with end-stage primary dilated or ischemic cardiomyopathy.
M.Mussur, A.Stasiak, J.Nożyński, M.Zembala, J.Kobos, W.A.Fogel

P35 Src protein involvement in histamine-induced MDA-MB-231 cells migration.
N. Mohamad, J. Porretti, P. Girardi, C. Cocca, E. Rivera, G. Cricco, G. Martín

P36 Modulation by histamine of β2 adrenoceptor-mediated cAMP accumulation in COS-7 cells: a possible allosteric action.
D.A. McNaught-Flores, M.A. Soriano-Ursúa, J.A. Arias-Montaño

P37 Radioprotective potential of histamine on rat small intestine and uterus.
E. Carabajal, N. Massari, M. Croci, D. Martinel Lamas, J.P. Prestifilippo, R.M. Bergoc, E.S. Rivera, V.A. Medina

P38 Comparison of the pharmacological and signalling properties of wild-type and A280V mutant human histamine H_3 receptors expressed in CHOK1 cells.
C. Flores-Clemente, A. Osorio-Espinoza, J. Escamilla-Sánchez, J.M. Arias-Montaño, J.A. Arias-Montaño

P39 The Th-1-associated cytokine CXCL10/IP-10 is downregulated in monocytes and myeloid dendritic cells – an effect possibly mediated via different histamine receptors in both cell types.
F. Jantzen, T. Werfel, R. Gutzmer

P40 Histamine inactivating enzymes in breast cancer cell lines.
D. Martinel Lamas, H.G. Schwelberger, E.S. Rivera, V.A. Medina

P41 Histamine actions in the normal human fibroblast cell line CCD-1059Sk.
J. Porretti, N. Mohamad, E. Badenas, M. Esnaola, E. Rivera, G. Martín, G. Cricco

11.45-13.00 Histamine – various aspects!

Chaired by Agnieszka Fogel and Kerstin Sander

11.45-12.00 **O18** Silencing human H_4R gene by novel triplex-forming molecule.
M. Falah , M. Azab, A. Michaeli, A. Rayan

12.00-12.15 **O19** HDC-GFP transgenic mouse.
H. Ohtsu, A. Sato, T. Moriguchi, J. Takai, M. Yamamoto

12.15-12.30 **O20** The histamine H_4 receptor is strongly expressed on a subset of Aδ sensory fibres at the level of the rat skin, DRG and dorsal horn of the spinal cord.
M. Katebe, P.L. Chazot

12.30-12.45 **O21** A multiapproach strategy to reveal the roles of histamine-related elements in rare diseases.
F. Sánchez-Jiménez, A. Pino-Ángeles, A. Reyes-Palomares, C. Acosta, J. Caro-Astorga, R. Castro-Oropeza, E. Melgarejo, J.L. Urdiales

12.45-13.00 **O22** Establishment of a histamine methods and tools database.
H.G. Schwelberger

13.00-14.00 Lunch and meeting of WG4

14.00-15.15 Young Investigator Award Oral Session and COST Update

Chaired by Perrti Panula and Bernie Gibbs

14.00-14.15 **O23** Role of histamine H_4R in bleomycin-induced pulmonary fibrosis.
A. Pini, AC Rosa, D. Bani, M. B. Passani, R. L. Thurmont, H. Stark, E. Masini (not part of competition)

14.15-14.30 **O24** Evidence supporting the existence of a novel histaminergic pathway in the regulation of experimental allergic encephalomyelitis susceptibility.
N. Saligrama, L. K. Case, R. Del Rio, R. Noubade, and C. Teuscher

14.30-14.45 **O25** The histamine 4 receptor blunts neutrophil degranulation by preventing beta 2 integrin-dependent signalling.
T. Perecko, V. Brown, D. Comer, R. Thurmond, K. Dib, M. Ennis

14.45-15.00 **O26** The anorexiant effect of oleoylethanolamide is modulated by neuronal histamine.
G. Provensi, L. Munari, P. Blandina, N. Galeotti, M.B. Passani

15.00-15.15 **O27** Sexual arousal, a role of histamine and orexins?
Y. Zhao, C. Anaclet, M. Perier, G. Guidon, C. Buda, T.H. Wang, J.S. Lin

15.15-15.45 Coffee and Poster viewing of the selected posters

15.45-16.00 **L7** "Progress and limitations in H_4R research"

Katherine Tiligada (University of Athens, Greece)
– COST Action BM0806

16.00-17.45 General Assembly of the EHRS

18.00-19.30 Meeting of the MC COST Action BM0806

20.00 Farewell Dinner

Abstracts of the Invited Lectures

L1 HISTAMINE H_4 RECEPTOR – LIGAND, BINDING AND ACTIVATION

H. Stark

The youngest member of the family of histamine receptor subtypes, the histamine H_4 receptor (H_4R), has received massive interest due to its influence on inflammatory and immunomodulatory processes.
With respect to drug development some different lead structures have already been described and at least one, perhaps more, have been advanced to clinical trials with humans. Despite this rapid progression there are still some pharmacological tools which are missing since the thus far adopted reference compounds give in different assays conflicting results. This clearly shows that we lack some understanding in the ligand interaction, the efficacy, and the cross-talk in this receptor.
Different classes of H_4R ligands will be discussed with an emphasis on the 2-aminopyrimidines which may have the highest number of compounds in this class in connection to strong variance in affinity as well as in efficacy, especially when different species being taken into account. Structurally strongly related 2-aminopyrimidines with comparable affinities, but large differences in efficacies have been investigated in molecular dynamic simulations proposing a different binding mode within this class of compounds depending on their efficacies as partial agonists or inverse agonists.
We propose a "pseudo ionic lock" motif in hH_4R that may affect receptor activation, but is not responsible for the high constitutive activity of the receptor. The latter may be caused by a salt bridge between transmembrane domains V and VI which can be seen as a widespread motif in aminergic GPCRs.
Kindly supported by the Hessian LOEWE programs NeFF, OSF and AFA as well as by the EU COST Action BM0806.
Werner T. et al. *ChemBioChem* **2010**, *11*, 1850-1855

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L2 INTERACTIONS BETWEEN MAST CELLS AND STRUCTURAL AIRWAY CELLS IN THE PATHOGENESIS OF ASTHMA

P. Bradding

Mast cells play a central role in orchestrating the complex pathophysiological processes underlying asthma and other allergic diseases. Mast cells present in the airways of chronic asthmatic subjects are present in a chronically activated state, with the ongoing release of a plethora of autacoid mediators, cytokines and proteases. These contribute to airway smooth muscle dysfunction, inflammatory cell recruitment and tissue remodelling. In asthmatic airways, mast cells infiltrate the airway epithelium, airway mucosal gland stroma, and the airway smooth muscle bundles. This therefore places activated mast cells in direct contact with these dysfunctional airway components. Recent *ex vivo* work using primary human airway cells has identified important bi-directional interactions between mast cells, airway smooth muscle cells and the airway epithelium. Manipulating the pathways which facilitate mast cell-structural cell cross-talk may offer novel approaches to the treatment of asthma and related allergic diseases.

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L3 LATE SIGNALING EVENTS FOR HISTAMINE RELEASE BY MAST CELLS

U. Blank

Mast cells are implicated in many chronic diseases with inflammatory components including allergy, asthma, atherosclerosis etc. and even cancer. Secretion of inflammatory products from preformed sources stored in cytoplasmic granules such as histamine represents a key step in the inflammatory process. Within the inflammatory site, the mast cell must integrate multiple and distinct activation signals for its secretory response. Therefore blocking signaling steps initiated by individual receptors is not a promising therapeutic approach in inflammation. By contrast, blocking secretion of its mediators by targeting the late signaling and vesicular trafficking steps is an attractive therapeutic strategy. In this talk, I will report on the progress in the molecular understanding and functional definition of the late signaling steps that lead to mast cell degranulation. In particular, I will focus on the mast cell secretory machinery that requires N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) to mediate membrane fusion and accessory regulators. The results show that the granule-localized SNARE Syntaxin3 and the fusion accessory effector Munc18-2 have complementary roles in degranulation. While Syntaxin 3 directly mediates fusion, its binding partner Munc18-2 controls granule translocation involving its capacity to interact with the microtubule cytoskeleton. Our results define a stimulatory Munc18-2 microtubule-dependent axis coupling the membrane fusion apparatus to receptor signaling and the cytoskeleton to facilitate SG translocation and SNARE-mediated membrane fusion.

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L4 EICOSANOIDS – PERFORMING LIPIDOMICS ON ACTIVATED MAST CELLS

G. Nilsson, S. Lundström, R. Saluja, J. Haeggström, C. Wheelock

Upon activation, mast cells have the capacity to release a wide variety of mediators, including histamine, proteases, eicosanoids and cytokines. Eicosanoids are made by oxidation of twenty-carbon essential fatty acids and are divided into four main families: the prostaglandins, prostacyclins, thromboxanes and leukotrienes. LTB4 and the cys-leukotrienes C4, D4 and E, as well as the prostaglandins D2 and E2 are well known mast cell mediators. The objective of this study was to investigate, with a global approach using lipidomics, the secretion of ninety lipid mediators representing the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) metabolic pathways, in two different mast cell populations (connective tissue-like (CTLMC) and mucosal-like (MLMC)), activated with the calcium ionophore A23187. Supernatants were taken 2, 8 and 15 min after activation and analyzed with LC-MS/MS. Twenty-eight oxylipins were found above the method limit of quantification in the CTLMCs and 27 oxylipins in the MLMCs. The most prominent differences included COX-derived dihomo-gamma-linolenic acid and arachidonic acid derived products which were all found in ~10 fold higher concentrations in the MLMCs following stimulation at all measured time points. Notable were also that the sum of 5-LOX mediated compounds (originating from dihomo-gamma-linolenic acid, arachidonic acid and eicosapentaenoic acid) were significantly higher in the MLMCs at 2 min ($p=0.008$), but significantly higher in the CTLMCs at 15 min ($p=0.02$). By interrogating the compounds individually and by using multivariate analyses it was apparent that in particular that AA derived 5-HETE, 5-KETE and the CysLTs most prominently followed this significant inverse shift in concentration over time. This study reveals the capacity of mast cells to secrete a high variety of oxylipins and also a difference in the ability between mast cells with different phenotypes.

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L5 THE ALLERGIC EFFECTOR UNIT: MAST CELLS – EOSINOPHILS INTERACTIONS REGULATE THE ALLERGIC RESPONSE

Francesca Levi-Schaffer, Moran Elishmereni

Mast cells and eosinophils, the key effector cells in allergy, co-localize particularly in the late and chronic stages of allergic inflammation. Recent evidence from our laboratory has outlined a specialized "allergic effector unit" (AEU) in which mast cells and eosinophils communicate via both soluble mediators and physical contact. Our objective was to evaluate the functional impact of this bi-directional crosstalk on the cells' survival and effector activities.
Human and murine mast cells and eosinophils were co-cultured under various conditions for a few hours or 1-3 days, and in selected experiments cell-cell contact was blocked. Cell survival, migration and mediator release were examined. Moreover flow cytometry was used to stain intracellular signaling molecules and surface receptors.
Mast cells significantly enhanced eosinophils survival both by soluble mediators and physical contact involving CD48-2B4 binding. Furthermore resting and IgE-stimulated mast cells led to eosinophil migration and activation through a paracrine-dependent mechanism. Eosinophils were found to enhance basal mast cells mediator release and IgE-mediated mast cells degranulation through CD48-2B4 interactions. Increased phosphorylation of activation-associated signaling molecules and enhanced release of tumor necrosis factor (TNF)-α were observed in long-term co-cultures. Eosinophils also showed enhanced expression of intercellular adhesion molecule (ICAM)-1, which depended on direct contact with mast cells.
In conclusion our findings describe a new role for mast cell/eosinophil interplay in augmenting short and long-term function in both cells, in a combined physical/paracrine manner. This enhanced functional activity may thus critically contribute to the perpetuation of the inflammatory response in allergic conditions.

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L6 H_4R AND NEUROINFLAMMATION: INSIGHTS FROM MOUSE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

M.B. Passani

The histaminergic system has been postulated to have a role in the pathogenesis of autoimmune diseases and there are several lines of evidence suggesting a key regulatory role of histamine in the widely used murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Therefore, strategies aimed at interfering with the histamine axis may have relevance in the therapy of CNS autoimmune disease. The H_4 receptor (H_4R) is distributed on immune cells and has a primary role in inflammatory functions. The H_4R has become a very attractive target for the treatment of asthma and autoimmune diseases[1], and the scientific community is displaying great excitement for the therapeutic potential for selective antagonists. Recent evidence has shown the topological and functional localisation of the H_4R also in the CNS of both humans and rodents[2,3]. However, the picture is more complex than expected, as recent data showed that the activation, and not the antagonism, of the H_4R leads to reduced pro-inflammatory capacity of at least dendritic cells in atopic dermatitis[4]. Results from our laboratory are also pointing in this direction, as H_4R antagonists tested in EAE murine model appear to exacerbate several parameters of the disease and up-regulate H_4R expression on T and dendritic cells in the spinal cord of EAE mice. Accordingly, H_4R KO mice also develop more severe EAE[5].
The complex and apparently controversial results summarized here, on the one hand provide compelling evidence that the H_4R has multiple roles in the function of various cellular elements that may be associated with immune disorders. On the other hand, these observations challenge the scientific community to develop the adequate treatments for different pathological conditions.

1 Bhatt et al., Mini Rev Med Chem 10, 1293-1308 (2010)
2 Connelly et al. Br J Pharmacol 157, 55-63 (2009)
3 Strakhova et al. Brain Res 1250, 41-48 (2009)
4 Gschwandtner et al. Immunology, 49-56 (2011)
5 del Rio et al. doi:10.4049/jimmunol.1101498 (2011)

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L7 PROGRESS AND LIMITATIONS IN H_4R RESEARCH

E. Tiligada

Histamine elicits pleiotropic actions largely through binding to four currently known G protein-coupled receptors (GPCRs), designated as H_1R-H_4R. The latest concept that histamine exerts immunomodulatory actions in inflammation through H_4R signalling and the potential exploitation of this activity for a range of poorly treatable chronic inflammatory diseases are currently under worldwide evaluation. Although the large body of evidence identifies the H_4R as a central player in initiating and propagating immune responses, the cell and tissue variability in H_4R-mediated signals and the profound intra- and inter-species differences in potency, selectivity and off-target effects of H_4R ligands hamper investigations and call for more cautious interpretation of H_4R-mediated effects *in vivo*. For instance, the complex pharmacology of H_4R ligands can be partly attributed to the functional selectivity exhibited by many GPCRs under different experimental or physiological environments. In this multifaceted system of immunoregulation further experimental approaches are needed to address numerous unresolved questions. For instance, what is the contribution of the H_4R in differentiating the phenotypes and chemotaxis of immunocompetent cells in inflammation? What are the molecular mechanisms underpinning H_4R cross-talk with immune-relevant pathways, such as TLR signalling and T_H1/T_H2 polarisation, and how would these interactions be useful in identifying more effective therapeutic targets for inflammatory diseases? Finally, it would be interesting to know whether autocrine or paracrine mechanisms are in operation considering that mast cells, the main histamine source in the body, express histamine receptors. Establishing the beneficial end-points of the histamine-mediated orchestration of the complex immune response is an altruistic challenge of EU RTD FP7 COST Action BM0806: Recent advances in histamine receptor H_4R research.

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Abstracts of the Oral Communications

O1 LIGAND-DIRECTED H_4R SIGNALLING: A STEP FORWARD TO OPTIMIZED H_4R DRUGS?

S. Nijmeijer[1], E.M. Rosethorne[2], H.F. Vischer[1], S.J. Charlton[2], R. Leurs[1]*

G protein-coupled receptors (GPCRs) acquired their name from the ability to activate G proteins to induce intracellular signalling. However, not only G proteins transduce GPCR activation, also other proteins such as β-arrestins can function as signalling scaffolds. Moreover, GPCR ligands do not always activate G protein- and β-arrestin-mediated signalling to an equal amount. This phenomenon is known as *ligand-directed signalling* and has been observed for various GPCRs.
The histamine H_4R was previously shown to couple to $G\alpha_i$ proteins, but has recently been added to the list of GPCRs that display ligand-directed signalling. The well-known antagonist JNJ 7777120 was surprisingly identified as a partial agonist in a PTX-insensitive PathHunter β-arrestin recruitment assay. In addition, JNJ 7777120 induced ERK phosphorylation in a time frame typical for β-arrestin-mediated signalling[$].
These observations led us to re-evaluate a variety of H_4R compound classes and to investigate their ability to induce PTX-sensitive CRE activity versus PTX-insensitive β-arrestin recruitment. We have identified compounds with a strong bias towards one of the tested pathways. The newly identified biased H_4R compounds will be useful pharmacological tools to study the functional consequences of biased H_4R signalling in future research.

[$]Rosethorne, E.M. & Charlton, S.J. *Mol Pharmacol*. 2011 Apr;79(4):749-57
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O2 INFLUENCE OF THE LIPOPHILIC PART OF 3-(1*H*-IMIDAZOL-4-YL)PROPYL CARBAMATES ON HISTAMINE H_3/H_4 RECEPTOR AFFINITY, SELECTIVITY, AND POTENCY

M. Więcek[1], T. Kottke[2], R. Seifert[3], H. Stark[2], K. Kieć-Kononowicz[1]

The human histamine H_4 receptor ($h$$H_4R$) has been identified independently by several research groups and is the newest member of the histamine receptor family. Histamine H_4Rs are preferentially active on hematopoietic and immune cells (e.g. eosinophils, mast cells, macrophages). [1] The $h$$H_4R$ is closely related to the human H_3R ($h$$H_3R$), therefore it is not surprising, that numerous imidazole-containing H_3R ligands have also significant affinity at the hH_4R [2]. Such compounds are considered as new pharmacological tools and could possess therapeutic profile in the therapy of inflammatory and immune disorders [3]. Recently we described branched alkyl, alkenyl and cycloalkyl carbamates of 3-(1H-imidazol-4-yl)propanol that showed both H_3R antagonist/inverse agonist activity and improved H_4R affinity [4, 5]. Expanding the investigation for histamine H_3 and/or H_4 receptor ligands in this class of compounds and structural requirements for affinity at these receptors, we introduced different substituents in the eastern part of the molecule.

The compounds were tested for their affinities at $h$$H_3R$, and at transiently expressed $h$$H_4R$ co-expressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$ subunits. Some compounds were also tested for their H_3 receptor potency *in vivo* after oral administration to mice. All tested compounds exhibited good affinity for the $h$$H_3R$ with K_i values in the range from 30 to 150 nM. Most compounds were also active *in vivo*. In respect to $h$$H_4R$ affinity in this group of compounds there are some without affinity to this receptor (selective for H_3R) and others with affinity in the micromolar concentration range (Ki = 600 - 4868 nM).These results indicated that selectivity for $h$$H_3R$ over $h$$H_4R$ among imidazole-derivatives is possible to achieve.

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[1] Zampeli E, Tiligada E. *Br. J. Pharmacol.* **2009**, *157*, 24 ; [2] de Esch IJP, et al. *Trends Pharmacol. Sci.*, **2005**, *26,* 462; [3] Tiligada, E., et al. *Expert Opin. Investig. Drugs* **2009**, 18, 1519; [4] Łażewska, D., *et al. *Bioorg. Med. Chem. Lett*. **2009**, 19, 6682; [5] Więcek M. et al. *Bioorg. Med. Chem.* **2011**, 19, 2850

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O3 DIFFERENTIAL BINDING KINETICS OF HISTAMINE H_4 RECEPTOR LIGANDS: FROM QUICK LEAVERS TO LONG-STAY BINDERS

H.D. Lim[1], S. Nijmeijer[2], H.F. Vischer[2], C. de Graaf[2], R.A. Smits[1], I.J.P. de Esch[1,2] and R. Leurs[1,2]

The histamine H_4 receptor is a G protein-coupled receptor associated with various inflammatory diseases such as allergic asthma, rheumatoid arthritis and pruritus. For this reason, H_4 receptor antagonists currently receive considerable attention as potential therapeutics. In our search for new H_4 ligands, several low affinity fragments were optimized to new high affinity H_4 receptor antagonists. Interestingly, study of the binding kinetics at the human H_4 receptor showed very different dissociative half-lives for several compounds. This could potentially explain part of the *in vivo* effectiveness of e.g. the reference antagonist JNJ 7777120. In conclusion, this study of H_4 ligands indicates important differences in H_4 receptor binding kinetics, which could ultimately be responsible for differences in *in vivo* activities.

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O4 HISTAMINE H_3 RECEPTOR REGULATES THE FUNCTIONS OF PANCREATIC β-CELL.

T. Nakamura, T. Yoshikawa, N. Noguchi, F. Naganuma, R. Harada, A. Mohsen and K. Yanai.

Histamine and its receptors in the central nervous system play an important role in energy homeostasis by modulating appetite and satiety. However, the effects of histamine on pancreatic β-cells in the islets of Langerhans, which secrete insulin to maintain glucose homeostasis, have not been elucidated.

First, we found histamine H_3 receptor (H_3R) was expressed on rodent and human pancreatic β-cells. Next, we examined the role of H_3R in glucose-induced insulin secretion (GIIS) from MIN6 cells, a cell line derived from mouse pancreatic β-cell. We revealed the inhibitory effect of H_3R on GIIS using pharmacological assays. In addition, H_3R signaling inhibited insulin granule exocytosis which was the final step in GIIS, because H_3R signaling did not affect the upstream events in GIIS such as the increase in intracellular ATP and Ca^{2+} concentration.

We found that the activation of H_3R with imetit attenuated BrdU incorporation in MIN6 cells. We examined which factors were involved in the H_3R-mediated inhibition of β-cell proliferation. Imetit decreased the phosphorylation of cyclic AMP response element binding protein (CREB) which was one of the essential transcriptional factors for ß-cell proliferation. These results indicated that H_3R signaling regulated β-cell proliferation by decreasing the CREB phosphorylation.

These lines of evidence might suggest that H_3R expressed in β-cells plays a pivotal role in energy homeostasis by regulating insulin secretion and β-cell proliferation. Therefore, H_3R will be an eligible diagnostic and/or therapeutic target for diabetic patients.
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O5 DIFFERENTIAL ROLE OF HYPOXIA-INDUCIBLE FACTOR-1 IN HUMAN MAST CELL AND BASOPHIL RESPONSES

B.F. Gibbs, I. Yasinska, V.V. Sumbayev

Hypoxia-Inducible Factor-1 (HIF-1) facilitates cellular adaptation to hypoxic conditions, which also occur during allergic inflammation, by controlling angiogenesis and glycolysis. Our aims were to address whether there is an overarching principle of HIF-1 involvement in controlling the release of histamine as well as the synthesis of angiogenic and inflammatory cytokines from human mast cells and basophils activated by IgE-dependent or innate immune triggers. Purified human basophils and LAD2 human mast were used for investigations of FcεRI and Toll-like receptor (TLR) ligand-induced responses. Real-time PCR, Western blotting, ELISA, fluorometry and luminometry were employed to assess the role of HIF-1 on the ability of these cells to release histamine, pro-allergic and angiogenic cytokines as well as to generate ATP. Mast cells expressed high background levels of HIF-1α, which were not significantly enhanced by IgE-, TLR-, or stem cell factor-mediated stimulation. Mast cell survival and cytokine synthesis was, however, markedly reduced by HIF-1alpha knockdown following both IgE- and TLR ligand stimulation. HIF-1 was also involved in IL-4 secretion from basophils caused by IgE-dependent triggering, but not by the TLR2 ligand PGN. In contrast to cytokine synthesis, histamine release in both basophils and mast cells was not controlled by HIF-1. We conclude that HIF-1 accumulation plays a crucial role in sustaining human allergic effector cell survival and function. This transcription complex facilitates the generation of both pro-angiogenic and inflammatory cytokines in mast cells, but has a differential role in basophil stimulation comparing innate immune stimuli with IgE-dependent triggering.

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O6 DESENSITIZATION OF IgE-DEPENDENT RESPONSES IN HUMAN LUNG MAST CELLS FOLLOWING EXPOSURE TO ANTI-IgE OR ANTIGEN.

A.Lewis, P.T. Peachell

Allergen immunotherapy has been employed clinically to desensitize allergic individuals to allergens. How allergen immunotherapy works has not been fully elucidated but a role for Treg cells, IL-10 and an isotype class switch from IgE to IgG have all been suggested. It is less clear what direct effects immunotherapy has on mast cells. The aim of this study was to determine the effects of long-term exposure of human lung mast cells to IgE-directed ligands on subsequent mast cell responses.

Mast cells were generated by disruption of lung tissue. Mast cells were further purified by flotation over Percoll gradients. In some experiments, cells were passively sensitized by incubation (24 h) with JW8-IgE, a nitrophenacetyl (NIP) specific IgE. To induce desensitization, cells were incubated (24 h) with or without anti-IgE or antigen (NIP-HSA) after which the cells were washed before challenge (25 min) with anti-IgE or antigen for histamine release. Histamine release was assayed by automated fluorometry.

Incubation (24 h) of mast cells with a maximal releasing concentration (2 µg/ml) of anti-IgE abolished the subsequent ability of mast cells to release histamine in response to anti-IgE. This anti-IgE treatment had no effect on the response of mast cells to the calcium ionophore, ionomycin. Overnight incubation of mast cells with a concentration of anti-IgE, 100-fold lower than maximal, also abolished the subsequent response to anti-IgE (2 µg/ml). Incubation with JW8-IgE led to a ~30% increase in the expression of IgE by mast cells as assessed by flow cytometry. Incubation (24 h) of JW8-IgE sensitized mast cells with a maximal concentration (10 ng/ml) of antigen abolished the subsequent response of mast cells to antigen but did not affect the response to anti-IgE.

These studies demonstrate that overnight exposure to anti-IgE or antigen desensitizes mast cells to IgE-directed activation. Moreover, antigen induces a 'homologous' form of desensitization.

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O7 ALLERGIC AIRWAY INFLAMMATION IN H_2R KNOCKOUT MICE: INCREASED SUSCEPTIBILITY INDEPENDENTLY OF TREG CELL NUMBERS

R. Ferstl, P. Konieczna, M. Ziegler, R. Frei, C.A. Akdis, L. O`Mahony

Greater than 15% of people in the western population suffer from allergy or asthma. In order to develop new and more effective therapies, a better understanding of the molecular mechanisms underpinning this chronic disease is required. Histamine, released by activated mast cells and immune cells, causes many of the symptoms associated with allergy and asthma. Histamine is recognized by 4 different Histamine receptors (H_1R-H_4R), each inducing its own signaling cascade. H_2R is known as an immune regulatory receptor but its role in allergy is not well described. We used a murine model of allergic airway inflammation similar to asthma to investigate the role of H_2R in this disease. For this female H_2R-/- mice and BALB/c wt mice were sensitized i.p. and OVA-aerosol challenged. Mice lacking H_2R showed significant increased cell numbers in bronchoalveolar lavages, mainly due to elevated eosinophil numbers. Lung histology confirmed increased inflammatory scores in knockout animals. Furthermore, *in vitro* re-stimulation with OVA induced higher Th1 and Th2 cytokine release from single cell suspensions from lungs, spleen and lymphnodes. All together, H_2R-/- mice develop more severe allergic airway inflammation. CD4+CD25+Foxp3+ Treg numbers were evaluated by flow cytometry in Peyer's patch, mesenteric lymph nodes, spleen, lung-draining lymph nodes, lung tissue and bronchoalveolar lavages. Treg numbers were similar in wildtype and knockout animals at all sites, with a tendency towards increased Treg numbers in H_2R-/- inflammed lung, perhaps related to altered anti-inflammatory compensatory mechanisms in the H_2R-/- animals.
In conclusion, H_2R is an important immunoregulatory receptor that influences the severity of allergic airway inflammation in murine models. In addition, the increased severity of disease was not associated with decreased Treg numbers suggesting that other immune cell populations may be directly influenced by histamine signaling through the H_2R.

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O8 HISTAMINE CONTRIBUTES TO HUMAN INFLAMMATORY JOINT DISEASE BY INCREASING THE RANKL/OPG RATIO THROUGH ALTERED NR4A ACTIVITY IN CHONDROCYTE CELLS

A.Gilmore , V. Marzaioli, H. Angerer, J.P. McMorrow, D. Zocco, A.N. McEvoy, M.H. Stradner, E.P. Murphy.

Histamine promotes immune complex-induced vascular leakage, a critical and early event that leads to joint specific autoimmune damage *in vivo*. Over-expression of histamine in synovial fluid, tissue and cartilage of rheumatoid arthritis (RA) and osteoarthritis (OA) patients support a central role of mast cells in perpetuating this inflammatory response. We aim to elucidate the histamine receptor-

mediated signaling pathways, transcriptional events and target gene expression in human chondrocyte cells.
Histamine receptor (H_{1-4}Rs) dependent regulation of nuclear transcription factors NR4A1-3; receptor activator of NF-κB-ligand (RANKL); and osteoprotegerin (OPG) mRNA levels were measured in human primary (n=8) and SW-1353 chondrocyte cells using QPCR and selective HR antagonists. sRANKL and OPG protein levels were determined by ELISA. NR4A protein levels and transactivity were evaluated by western, immunocytochemistry and luciferase reporter assays. Stable depletion of NR4A1-3 was achieved by lentiviral transduction of NR4A shRNA.
Primary human chondrocytes express differential steady state levels of H_{1-4}R mRNA. Histamine, in a time- and concentration-dependent fashion, modulates expression of NR4A1-3. Functional studies using HR antagonists reveal that histamine selectively signals through H_1R and H_2R to modulate RANKL and NR4A2 expression, with modulation significantly reduced in cells pre-treated with inhibitors of NF-κB, PKA, MAPK and MEK1/2 signalling pathways. Histamine robustly modulates the expression of RANKL, with modest effects on OPG, leading to significantly increased RANKL/OPG mRNA and protein ratios. Stable knockdown of NR4A1-3 expression results in reduced endogenous OPG levels and the loss of histamine-dependent regulation of RANKL expression.
In conclusion histamine, *via* H_1R and H_2R, may contribute to the development of inflammatory joint disease by enhancing the expression ratio of RANKL/OPG through altered NR4A activity, in human chondrocyte cells.
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O9 HISTAMINE DOWNREGULATES SKIN BARRIER PROTEINS – A POTENTIAL ROLE IN THE PATHOGENESIS OF ATOPIC DERMATITIS

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Patients with atopic dermatitis have defects in epidermal keratinocyte differentiation and skin barrier function. These defects result either from inherent alterations in the expression of differentiation-associated proteins and/or the influence of inflammatory mediators on terminal keratinocyte differentiation. Skin mast cells participate in skin inflammatory reactions via the release of various soluble mediators, most prominently histamine. Here we studied the effect of histamine on epidermal keratinocyte differentiation in monolayer cultures, in an *in vitro* organotypic skin model and in human skin explant cultures. The expression of differentiation-associated proteins was assessed by quantitative realtime-PCR, Western blot and immunofluorescence labeling. Histamine reduced the expression of filaggrin, loricrin, keratin 1 and keratin 10 by up to 90% in keratinocyte monolayer culture and in human *in vitro* skin models. The influence of histamine on the expression of differentiation associated proteins was dose-dependent and only detectable when histamine was added to the cultures before the onset of keratinocyte differentiation. Accordingly, we did not find a change in the expression of differentiation-associated proteins in intact human skin biopsies after *ex vivo* treatment with histamine. Furthermore, addition of histamine to organotypic skin cultures reduced the expression of the cell-cell contact proteins corneodesmosin and occludin and perturbed the inside-out barrier function of the epidermis. Taken together these findings suggest an important role of histamine as deregulator of epidermal keratinocyte differentiation, contributing to the sustained skin barrier defects observed in atopic dermatitis.

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O10 BLOOD HISTAMINE LEVELS AND SKIN H_4R EXPRESSION IN PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA

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Chronic spontaneous urticaria (CU) is a common skin disorder characterized by histamine (HA) release from activated skin mast cells and basophils. An underlying cause remains elusive and symptomatic therapies often lack efficacy, thus urging the need for the identification of more beneficial therapeutic strategies. Considering the recent concept of the immunomodulatory role of the HA H_4 receptor (H_4R), this study aimed to assess the relationship of blood HA levels and skin H_4R expression to the response of CU patients to standard therapy. Peripheral blood samples were collected from adult male (n=3) and female (n=10) CU patients of 45±12 years of age, unresponsive to anti-H_1R drugs (group A) and with full remission of urticarial lesions during anti-H_1R treatment (group B). Whole blood and serum HA levels were determined fluorometrically and presented as ng/ml of blood. H_4R expression was detected immunohistochemically on puncture biopsies (4 mm) from the urticarial lesions. Whole blood HA levels were significantly higher ($p<0.01$) in patients with refractory CU (30.7±12.3, n=9) compared to responsive subjects (13.3±2.3, n=4). In contrast, serum HA levels were comparable in the two groups, being 7.7±0.8 and 9.1±1.3 for group A and B, respectively. Interestingly, a significant correlation ($r<0.05$) between whole blood HA levels and monocyte counts was observed in group A samples. Prominent anti-*h*H_4R immunoreactivity was detected in the skin biopsies, with no apparent change in total skin sample extracts. However, representative samples from the two groups showed differential anti-*h*H_4R expression profiles. These data provide the first evidence linking whole blood HA levels and monocyte counts in refractory CU. The differential H_4R expression profile in skin biopsies from responsive and unresponsive patients points to a probable role of the H_4R in CU that deserves further consideration.

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O11 ANTAGONISM OF THE HISTAMINE H_4 RECEPTOR REDUCES LPS-INDUCED TNF PRODUCTION IN VIVO

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Antagonism of the histamine H_4 receptor (H_4R) has been shown to be anti-inflammatory in a number of preclinical disease models, however the exact mechanisms behind this are still being uncovered. *In vitro*, the receptor has been shown to interact with TLR and impact inflammatory mediator production from a number of different cells types. Here it is shown that this interaction can also occur in vivo. Two different H_4R antagonists, JNJ 7777120 and JNJ 28307474, inhibited LPS-induced TNF production in mice. Furthermore, this production was also reduced in H_4R-deficient mice. TNF mRNA analysis showed that the major source of the cytokine was the liver and not blood, and that the H_4R antagonist only reduced the expression levels in the liver. The inhibition was only observed with *in vivo* administration of both the H_4R antagonists and LPS suggesting that the effect is mediated by tissue resident cells. In support of this conclusion, depletion or inactivation of macrophages reduced the TNF levels and eliminated the H_4R sensitivity. Treatment with an H_4R antagonist also reduced LPS-induced liver injury and blocked LPS-enhanced lung inflammation in

mice. In conclusion, the data support an interaction between H_4R and TLR activation *in vivo* which can drive inflammatory responses.

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O12 THERAPEUTIC POTENTIAL OF H_4R AGONISTS IN AN EXPERIMENTAL MODEL OF HUMAN BREAST CANCER

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We have previously reported the presence of the histamine H_4 receptor (H_4R) in benign and malignant lesions and cell lines derived from the human mammary gland. The H_4R is one of the main subtypes responsible for the histamine-induced responses in MDA-MB-231 breast cancer cells. The aim of the present work was to evaluate the effects of H_4R ligands on the survival, tumor growth rate, metastatic capacity and molecular pattern of expression of antigens related with the proliferative and apoptotic potential in a breast cancer experimental model. For that purpose, we established orthotopic xenograft tumors of the highly invasive human breast cancer cell line MDA-MB-231 in immune deficient nude mice. We employed the following H4R agonists: histamine (1 and 5 mg/Kg, sc), clozapine (1 mg/Kg, sc), and the experimental compound JNJ28610244 (10 mg/Kg, sc).
Results indicate that developed tumors were highly undifferentiated and that the H_4R was the major histamine receptor expressed. They also exhibited high levels of histidine decarboxylase, histamine content and proliferation marker (PCNA) while displaying low levels of apoptosis. Mice of the untreated group displayed a median survival of 60 days, and a tumor doubling time exponential growth of 7.4±0.6 days. A significant decrease in tumor growth evidenced by an augmentation of the tumor doubling time was observed in H_4R agonist groups (13.1±1.2, $P<0.01$ in histamine group; 15.1±1.1, $P<0.001$ in clozapine group; 10.8±0.7, $P<0.01$ in JNJ28610244 group). This effect was associated with a decrease in the expression levels of the H_4R (6.6% vs. 29.0%, $P<0.05$) and of the PCNA (64.3% vs. 82.8%, $P<0.05$). Histamine treatment significantly increased median survival (80 days; Log-rank Mantel-Cox Test, $P=0.0025$; Gehan-Breslow-Wilcoxon Test, $P=0.0158$). We conclude that histamine through the H_4R exhibits a crucial role in tumor progression. Therefore, H_4R ligands offer novel therapeutic potential as adjuvants for breast cancer treatment.
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O13 WAKING ACTION OF URSODEOXYCHOLIC ACID DEPENDS ON HISTAMINE AND $GABA_A$ RECEPTOR BLOCK

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Since ancient times ursodeoxycholic acid (UDCA), a constituent of bile, is used against gallstone formation and cholestasis. We show now that UDCA promotes wakefulness during the active time of the day, lacking this activity in histamine-deficient mice. In cultured hypothalamic neurons UDCA synchronized the firing, an effect not present in the presence of the $GABA_AR$ antagonist gabazine. In histaminergic neurons recorded in slices, UDCA reduced amplitude and duration of spontaneous and

evoked inhibitory postsynaptic currents (IPSCs) but left the firing unchanged. In acutely isolated histaminergic neurons UDCA blocked GABA-evoked currents and sIPSCs starting at 10µM (IC_{50}=70µM), and did not affect NMDA- and AMPA-receptor mediated currents at 100µM. Recombinant $GABA_A$ receptors composed of α1, β1-3 and γ2L subunits expressed in HEK293 cells displayed similar sensitivity to UDCA as native $GABA_A$ receptors. The mutation α1V256S known to reduce the inhibitory action of pregnenolone sulphate reduced the potency of UDCA. The mutation α1Q241L, which abolishes $GABA_AR$ potentiation by several neurosteroids had no effect on $GABA_AR$ inhibition by UDCA. In conclusion, UDCA enhances alertness through disinhibition of the histaminergic system

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O14 LACK OF EFFECTS BY ABT239, A HISTAMINE H_3 RECEPTOR ANTAGONIST, IN HISTAMINE-DEFICIENT ANIMALS

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Reports of improved cognitive functions with H_3 receptor (H_3R) antagonists in animals prompted their use in the clinic for the potential treatment of cognitive impairments associated with Alzheimer's disease, Parkinson's disease, schizophrenia and ADHD. H_3R antagonists increase cortical ACh release and this effect may relate to their precognitive properties. Since H_3Rs function as auto- and hetero-receptors that restrict histamine and ACh release, whether H_3R antagonists require an intact histamine neuronal system to improve cognition is an important question. Therefore, we evaluated the effects of ABT239 (3 mg/kg,) i.p. administration to histidine decarboxylase (HDC)-KO mice, which are unable to synthesize histamine, or wild type (WT) littermates on the Object Recognition Test. In a test given 24 h after the first trial, ABT239-treated WT mice spent significantly more time exploring novel rather than familiar objects, whereas saline-injected WT mice did not, nor did HDC-KO mice. Also acutely HA-depleted CD1 mice by means of i.c.v. injection of α-fluoromethyl-histidine (α-FMH, 5µg.), an irreversible inhibitor of HDC, failed to respond to ABT239 in the same test. To learn the correlation with the cholinergic tone, we implanted adult, male SD rats with two contralateral microdialysis probes in the pfCX and measured ACh and histamine release by HPLC-electrochemical or -fluorometric detection. Animals received an i.c.v. injection of saline or α-FMH (5 µg). Both probes were perfused with Ringer's solution (2µl/min) and 15-min samples were collected. In the controls, ABT239 (3 mg/kg, i.p.) elicited significant increases (2-3 times) of HA and ACh basal releases (ACh 620±60 fmol/15min; HA 50±5 fmol/15min; ANOVA/Bonferroni). α-FMH-treated rats displayed both ACh and HA release below detectable sensitivity before and after ABT239 injection. These data suggest that ABT-239 requires the integrity of histamine neuronal system to exert its effects.

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O15 COMBINATORIAL ROLES FOR HISTAMINE H_1-H_2 AND H_3-H_4 RECEPTORS IN AUTOIMMUNE INFLAMMATORY DISEASE OF THE CENTRAL NERVOUS SYSTEM

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system in which histamine (HA) and its receptors have been implicated in disease pathogenesis. HA exerts its effects through four different G protein-coupled receptors designated H_1-H_4. We previously examined the effects of traditional single HA receptor (HR) knockouts (KO) in EAE, the autoimmune model of MS. Our results revealed that H_1R and H_2R are propathogenic, while H_3R and H_4R are anti-pathogenic. This suggests that combinatorial targeting of HRs may be an effective disease modifying therapy (DMT) in MS. To test this hypothesis, we generated H_1H_2RKO and H_3H_4RKO mice and studied them for susceptibility to EAE. Compared to wild-type mice, H_1H_2RKO mice developed a less severe clinical disease course whereas the disease course of H_3H_4RKO mice was more severe. H_1H_2RKO mice also developed less neuropathology and disrupted blood brain barrier permeability compared to WT and H_3H_4RKO mice. Additionally, splenocytes from immunized H_1H_2RKO mice produce less IFN-γ and IL-17. These findings support the concept that combined pharmacological targeting of HRs may be an appropriate ancillary DMT in MS and other immunopathologic diseases.

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O16 HUMAN ASTROCYTES TRANSPORT HISTAMINE THROUGH PLASMA MEMBRANE MONOAMINE TRANSPORTER

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The removal of extracellular histamine from the synaptic cleft is an essential process for terminating the signal transduction. Previous studies using rodents revealed that histamine was mainly transported and metabolized in astrocytes. However, the histamine transport activity of human astrocytes and the mechanism of histamine uptake remained to be elucidated.

We first examined histamine uptake in normal human astrocytes using [^{3}H]-histamine. Histamine was taken up into the cells in a time- and dose-dependent manner. The values of Km and Vmax were high and the uptake was not dependent on the extracellular concentration of Na^+/Cl^-, suggesting that a low-affinity/high-capacity and Na^+/Cl^-- independent transporter(s) is responsible for histamine transport in human astrocytes. Histamine is reported to be a substrate for three low-affinity/high-capacity and Na^+/Cl^--independent transporters: organic cation transporter 2 (OCT2), OCT3 and plasma membrane monoamine transporter (PMAT). The inhibition assays using various reagents indicated OCT2 was not involved in the histamine transport, because tetraethylammonium, a substrate for OCT2, did not inhibit histamine transport in human astrocytes. RT-PCR analysis revealed that *PMAT* was most highly expressed in human astrocytes, and *OCT2* and *OCT3* were barely detected. Furthermore, PMAT knockdown using siRNA resulted in a remarkable reduction of histamine uptake with a corresponding decrease in *PMAT* expression.

In the present study, we clearly demonstrated that human astrocytes had sufficient ability for histamine transport and that PMAT played a predominant role in histamine transport by human astrocytes. These findings indicate that histamine transport through PMAT in human astrocytes is involved in the regulation of extraneuronal histamine concentration and the activity of histaminergic neurons.

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O17 DYNAMIC REGULATION OF HISTAMINERGIC AND DOPAMINERGIC NETWORKS IN ZEBRAFISH BRAIN

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Histamine regulates motor functions through interactions with the dopaminergic system via at least striatal and nigral mechanisms. In Parkinson's disease (PD), histamine levels are increased selectively in the striatum and substantia nigra. However, it has been unclear if this is due to the PD process, treatment with L-DOPA and other drugs, or if it is an important factor in disease pathogenesis. Histamine is also important in Tourette syndrome, where *hdc* mutation is linked to the disease and H_1 receptor pathway changes are found. We have established methods to study detailed changes in modulatory transmitters in zebrafish, a species in which one can count all neurons with specific markers. Translation inhibition of tyrosine hydroxylase 1 (TH1) caused a locomotor abnormality, decline in dopamine, noradrenaline and adrenaline levels. Translation inhibition of tyrosine hydroxylase 2 (TH2), which is expressed in the close vicinity of the histaminergic neurons, caused a decline in dopamine levels and an increase in *hdc* mRNA and number of histaminergic (*hdc* mRNA expressing and histamine immunoreactive) neurons. In agreement with a concept of bidirectional regulation of the histamine system by dopaminergic input, L-DOPA and dopamine receptor agonists downregulated the number of histaminergic neurons. The results suggest that L-DOPA or dopamine produced by TH2 neurons exerts trophic effects on histaminergic neurons in the brain.

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O18 SILENCING HUMAN H_4R GENE BY NOVEL TRIPLEX-FORMING MOLECULE

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Over the past two decades, there has been considerable interest in developing novel chemicals that can specifically silence a gene and inhibit its expression. Any approach that can specifically and efficiently suppress the expression of genes is recognized to be highly valuable. Two approaches that have received much attention are antisense oligonucleotides and siRNA. Both approaches target the RNA product of the expressed gene and in due course result in destruction of this target. An alternative approach that directly targets and inhibits gene expression utilizes triplex-forming molecules (TFMs). One advantage of this strategy is the relatively few DNA molecules that need to be targeted (usually 1 or 2 per cell), allowing for dosage reductions that lower toxicity for patients. This provides a distinct advantage over targeting RNA molecules, which are continuously produced when the gene is transcribed, requiring the antisense oligonucleotide or siRNA to be constantly present in the cell. Targeting DNA directly also prevents biofeedback loops from becoming effective drug resistance mechanisms.

Based on our proprietary bioinformatics tools, we have designed a highly specific molecule that tightly binds a selected DNA sequence along the hH_4R gene, stopping its RNA transcription. This novel synthetic molecule which target the hH_4R gene (hH_4R-TFM) is highly selective and designed not to interact with non-targeted sequences along the human genome. Our TFMs are chemically engineered to bind to the major groove of the targeted DNA fragment. Our molecules interact with the major groove by forming a stable Hoogsteen interaction. This mode of binding is significantly different from those of antisense oligonucleotides or siRNAs which bind nucleic acids via base-pairing.

The H_4R-TFM binds efficiently with its DNA target at physiological temperature and pH, prevents amplification in RT-PCR and can be used to disrupt *in* V*itro* transcription and prevent replication *of DNA* containing a binding site for the hH_4R-TFM.

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O19 HDC-GFP TRANSGENIC MOUSE

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In vivo activity of histamine has been studied well enough to stimulate discussion in the last decade, partly because of the generation of various kinds of gene manipulated mice. On the contrary, the source of histamine in physiological and pathological situation has not been studied as well, partly because of the lack of useful reporter mice.
We aimed to prepare the reporter mice for histidine decarboxylase gene by using a BAC construct to mimic the expression of the gene. It will reduce the labour and difficulty of identification of gene-expressing cells.
We used the longest BAC clone, RP23-40N15, in a library of CHORI for HDC gene. This HDC-BAC clone was homologously recombinated with the plasmid containing HDC-promoter, GFP gene, HDC gene without 1st exon, and 3' region in E. coli strain EL250. Isolated BAC DNA was injected into a fertilized egg to produce a BAC transgenic mouse.
FACS analysis of peritoneal cells of the transgenic mice showed GFP high expressing cells in the Mac1- and/or c-kit-positive cell fraction indicating that peritoneal macrophages and/or mast cells were positive for GFP expression.
Newly generated BAC-based transgenic mice are promising reporter mice to observe the transcriptional expression of HDC gene. The transgene contains the longest franking regions of HDC gene without 1st exon.

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O20 THE HISTAMINE H_4 RECEPTOR IS STRONGLY EXPRESSED ON A SUBSET OF Aδ SENSORY FIBRES AT THE LEVEL OF THE RAT SKIN, DRG AND DORSAL HORN OF THE SPINAL CORD

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The histamine H_4 receptor (H_4R) is the most recent receptor identified belonging to the histamine receptor subfamily of G -protein coupled receptors (GPCR). Until recently the H_4 receptor was thought to be largely expressed on haematopoietic cells and as a result, is a possible key new target for inflammatory disorders [1].
Immunohistochemical studies from our laboratory and others [2] have revealed strong H_4R expression on the soma and some processes of cervical and lumbar dorsal horn sensory neurons with more intense staining on small and medium diameter cells, and subpopulations of dorsal root ganglia, suggesting a potential role in nociception and a new target for the modulation of chronic inflammatory pain disorders. The H_4R ligand, JNJ7777120, exhibits profound antipruritic, and analgesic effects in the Freund's Complete Adjuvant (FCA) chronic pain model [3], the latter where spinal NMDARs are hyperactivated (Chazot et al., unpublished data). Our hypothesis was that the H_4R would be expressed exclusively on C-fibres.
We have used a double immunofluoresence labelling approach to determine the possible co-localisation of H_4Rs on C- (Substance P) and Aδ nociceptive fibres (CGRP) at the level of skin, dorsal

root ganglia (DRG) and the dorsal horn neurones of the spinal cord in the rat. At all levels of the sensory pain pathway tested, our results surprisingly show expression on both C-fibres, particularly at the level of the skin and DRGs, and modestly at the level of the spinal cord, and prominently on a subset of Aδ fibres, at all levels. H_4R sensory neuron expression, as with the H_3R [4], were detectable at the level of the periphery in the skin dermis.

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O21 A MULTIAPPROACH STRATEGY TO REVEAL THE ROLES OF HISTAMINE-RELATED ELEMENTS IN RARE DISEASES

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Alterations in the HIS-related elements have been found in widely spread human pathologies (anaphylaxis and other inflammatory responses, peptic ulcer, neurological and cardiovascular disorders and cancer progression, etc). From all of these reasons, HIS could be considered the most versatile biogenic amine, playing many different (and sometimes antagonistic) roles in mammalian physiology. Rare diseases (RDs) are very low-prevalence pathologies (<2 patients/1000 inhabitants). More than 6000 RDs (>20 x 10^6 patients in Europe) have been described. One of the major problems for RD therapy is the lack of interest to invest in R&D on drugs useful for a small number of patients. Inflammation and neurological abnormalities are commonly included as causes or symptoms of many RDs. The initial hypothesis underlying this work was that HIS must be involved in causes/symptoms of many RDs. In fact, HIS-related genes accomplish the most common properties for typical disease related genes (Goh et al., 2007). Systems Biology resources, text mining technologies, metabolic and molecular modeling, combined with experimental validation, provide an efficient way to extract emergent knowledge from the integration of previous fragments of information. By applying this strategy, we are developing several studies based on biocomputational-driven hypotheses with the aim to unveil the putative usefulness of HIS metabolism/signaling modulators in diagnosis, prevention or therapy of these identified RDs. In addition to basic new information on histidine decarboxylase dimerization and its usefulness for selective intervention of inflammatory and neurological RDs, interesting information have emerged on the putative involvement of histamine in several RDs, for instance, Tourette and Sotos syndromes, among others.

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022 ESTABLISHMENT OF A HISTAMINE METHODS AND TOOLS DATABASE

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COST Action BM0806 Recent Advances in Histamine Receptor H_4R Research brings together scientist and industrial partners from many different disciplines who provide enormous expertise in their

respective field. In order to make this expertise transparent and usable for all action members and for histamine researchers in general one goal of Working Group 1 is to establish and run an internet database covering all methods and tools available at participating institutions.
In the first phase to build this database, a simple questionnaire was used to collect, evaluate and combine information on available methods and tools from individual participants. This methods and tools overview will serve as a contents and reference point for users. In the second phase, detailed protocols and descriptions of tools are collected from individual contributors and made available for users in a common, easy to use format. In the third phase, the database will be constantly updated with new information and errors will be corrected based on user feedback.
In its final version, this histamine methods and tools database should provide a comprehensive collection of all available methods (techniques, assay systems, cell systems, animal models, patient based studies), include ready-to-use protocols, give contact information for method based inquiries (technical help), inform about sources of critical tools (antibodies, reagents, compounds), and finally identify areas where new methods, techniques or tools should be developed.

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O23 ROLE OF HISTAMINE H_4R IN BLEOMYCIN-INDUCED PULMONARY FIBROSIS

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Pulmonary fibrosis is a progressive and lethal illness characterized by inflammation and abnormal remodeling of lung parenchyma. No cure exists for this disease. There are various models used for the study of pulmonary fibrosis, among these, the bleomycin one is the best characterized murine model in use. Bleomycin alters oxidant/antioxidant balance and ROS overproduction activates several intracellular signaling pathways, leading to pro-inflammatory cytokine production. The histamine H_4R, expressed on cell of immune origin, plays an important role in inflammatory process. We previously demonstrated that JNJ7777120 (JNJ), a selective H_4R antagonist, potentiates the beneficial effect of naproxen in this experimental model. The aim of the present study was to investigate the role of different H_4R ligands in controlling inflammation and pulmonary fibrotic process induced by bleomycin. C57/bl6 mice were treated with vehicle, JNJ (total dose 40 mg per Kg/bw) or ST-1124 (partial inverse agonist), ST-1006 (partial agonist) and ST-994 (neutral antagonist) at equimolar doses, released by micro-osmotic pumps for 21 days. Airway resistance to inflation, an index of lung stiffness, was assayed and lung tissue processed to evaluate inflammation and fibrosis. Our results indicate that JNJ and ST-994 exert an anti-inflammatory effect, as shown by the significant decrease in the levels of PGE_2, MPO, an index of leukocyte infiltration, and TBARS, markers of oxidative stress. They also reduce the relative number of goblet cells, the thickness of smooth muscle layer (parameters of inflammation-induced adverse bronchial remodeling), the level of pro-fibrotic cytokine (TGF-β) and collagen deposition; these effects are accompanied by a decrease in airway resistance to inflation. Our results indicated that H_4R blockade is associated with an anti-inflammatory and anti-fibrotic effect and may offer a new therapeutic option for the treatment of Th2-dependent lung inflammatory disease.

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O24 EVIDENCE SUPPORTING THE EXISTENCE OF A NOVEL HISTAMINERGIC PATHWAY IN THE REGULATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS SUSCEPTIBILITY

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Histamine (HA) is a key regulator of experimental allergic encephalomyelitis (EAE), the autoimmune disease model of multiple sclerosis (MS). Histidine decarboxylase deficient mice (HDCKO), which are unable to synthesize HA, exhibit more severe EAE and increased IFN-γ production by splenocytes in response to myelin oligodendrocyte glycoprotein 35-55. HA exerts its effects through four different G protein coupled receptors (GPCR): H_1, H_2, H_3 and H_4 (H_{1-4}R). Each HA-receptor has been shown to influence EAE pathogenesis. In the mammalian brain, however, there is evidence for the existence of non-GPCR signaling by HA which is picrotoxin-sensitive and mediated by chloride conductance. In addition, γ-aminobutyric acid ($GABA_A$) receptor subunits can form HA-gated chloride channels *in vitro* suggesting that an ionotropic HA-receptor might contain known ligand-gated chloride channel subunits. To test the hypothesis that non-GPCR signaling by HA plays a role in immune responses, we generated H_{1-4}RKO mice and studied their susceptibility to EAE. Here we report that in contrast to HDCKO mice, H_{1-4}RKO mice develop less severe EAE compared to wild-type animals. Furthermore, splenocytes from immunized H_{1-4}RKO mice produce significantly less IFN-γ compared to WT mice. Taken together these data support the existence of a novel HA signaling pathway in regulating EAE susceptibility.

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O25 THE HISTAMINE 4 RECEPTOR BLUNTS NEUTROPHIL DEGRANULATION BY PREVENTING BETA 2 INTEGRIN-DEPENDENT SIGNALLING

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Histamine is a biogenic amine implicated in allergic asthma. In the airways, its main targets are eosinophils, smooth muscle and the endothelium. There are 4 types of histamine receptors (H_1R, H_2R, H_3R, and H_4R). All belong to the G protein-coupled receptor family. Histamine has also been shown to regulate the functions of other immune cells, including neutrophils. However, little is known on the nature of the histamine receptors involved in the regulation of neutrophil functions.
We investigated whether the H_4R regulates beta2 integrin-dependent adhesion and degranulation in human neutrophils.
Neutrophils were isolated from blood by Dextran sedimentation and centrifugation through Ficoll-Hypaque. Neutrophils adherent to fibrinogen (a ligand for beta2 integrins) were stained with crystal violet and the OD of the eluted dye was read using a spectrophotometer. Degranulation was assessed by ELISA by measuring the release of lactoferrin.
The chemoattractant fMLP (10^{-7} M) induced a beta2 integrin-dependent adhesion and degranulation of human neutrophils. In contrast, histamine by itself did not have such an effect. However, histamine (10^{-6} M) blocked fMLP-induced adhesion-dependent degranulation of human neutrophils by ca. 60 %. The H_4R agonist JNJ28610244 (10^{-5} M) reduced release to ca. 38 % of fMLP alone. Moreover, the H_4R antagonist JNJ7777120 prevented the inhibitory effect of histamine on neutrophil degranulation. Interestingly, neither histamine nor the H_4R agonist JNJ28610244 blocked fMLP-induced beta2 integrin-dependent neutrophil adhesion indicating that histamine did not interfere with the expression or change of conformation of beta2 integrins induced by the chemoattractant fMLP.

Our results demonstrate for the first time that the H_4R blocks the neutrophil's anti-microbial functions and that this effect is mediated through inhibition of beta2 integrin-dependent outside-in signalling (p38 MAP Kinase).
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O26 THE ANOREXIANT EFFECT OF OLEOYLETHANOLAMIDE IS MODULATED BY NEURONAL HISTAMINE

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The modulatory effect of both brain histamine (HA) and Oleoylethanolamide (OEA) on feeding behaviour is robust, but the mechanisms involved are not clear and nothing is known about the temporal or causal relationship between HA and OEA in controlling food intake. To learn if OEA affects feeding behaviour via the histaminergic system we measured food consumption in normal and genetically or pharmacologically HA-deprived mice. Mice were food deprived for 12h and then received pharmacological treatments. Food consumption was measured every 15 min for the 1st h and at increasing intervals for the following 24 hrs. OEA-treated (10 mg/kg, i.p.) WT mice eat significantly less than vehicle-treated WT mice. However, the anorexiant effect of OEA was attenuated in HDC-KO mice. Accordingly, the anorexiant effect of OEA was also attenuated in acutely HA-depleted CD1 (through i.c.v. injection of 5µg of α-fluoromethyl-histidine), but not in control mice. On the other hand, the treatment with the H_3R antagonist ABT239 (3 mg/Kg, i.p.) potentiated the effect of OEA (5 mg/kg, i.p.). In order to investigate if changes in neuronal activation are related with the behavioral differences, we evaluated the c-Fos expression in HDC-KO and WT mice using immunohistochemistry in brain areas involved in feeding behavior: Infralimbic Cortex (IL-Cx), Nucleus Accumbens (Nacc) and Paraventricular Nucleus (PVN). Mice were fasted for 12h and then treated with OEA (10 mg/kg, i.p.) or vehicle and sacrificed after 2 hrs. OEA treatment decreased the number of c-Fos^+ cell nuclei in the Nacc of both HDC-KO and WT mice. No changes were observed in the IL-Cx of either genotypes. However, in the PVN an increase of c-Fos expression was observed in OEA-treated WT but not in HDC-KO mice. Our results indicate that the histaminergic system is involved in the anorexiant effects of OEA and that disregulation of PVN neuronal activity may be responsible for the partial lack of efficacy of OEA in HA deficient mice.

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O27 SEXUAL AROUSAL, A ROLE OF HISTAMINE AND OREXINS ?

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Sexual arousal ensures the necessary waking state allowing the anticipation and performance of sexual activities and so is a prerequisite for reproduction. We hypothesize that a full expression of such a behavioural state requires highly optimal convergent and divergent activities of the brain arousal systems notably histamine (HA) and orexins (Ox). To test our hypothesis, wild type (WT) and histidine-decarboxylase (HDC) KO mice were chronically implanted for EEG and sleep-wake monitoring under baseline conditions (12h light/dark cycle) and during the sexual arousal test, which consisted of introducing over a 4h period, a female mouse into the habitual cage of a male mouse, the two mice being separated by a transparent plexiglass with a maximal number of holes (diameter =14-16 mm), allowing the physical contacts, but prevents their copulation. Placement of a male

mouse was used as control. The test was performed both during light and dark phases. We found that the presence of a female mouse elicited in the male a significant increase in waking (+49 % vs baseline lightness, n=32, p<0.001). This increase is not significant if another male was introduced (+10% vs baseline, n=32, p=0.2). This sexual arousal appeared to depend on sexual hormones because ovariectomized female mice or those pretreated with Tamoxifen (an estrogen receptor antagonist) did not elicit significant sexual arousal in male animals and because male mice pretreated with Flutamide (an androgen receptor antagonist) did not show any additional waking in the presence of a female. Secondly, acute application of α-FMH (a specific inhibitor of HA synthesis) or SB-334867 (Ox1-receptor antagonist) both markedly impaired the sexual arousal in the male mice. Finally, whereas the sexual arousal appeared intact in HDC KO mice, that of Ox KO mice remained to be determined. These data indicate that both the HA and Ox systems, probably driven by sexual hormones, participate in the promotion of sexual arousal and that chronic HA loss could be compensated by up-regulated compensatory mechanisms.

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Abstracts of the Poster Presentations

P1 UNDERSTANDING HOW HISTAMINE INDUCES NEURON DIFERENTIATION

G. Rodríguez, I. Velasco, G. García-López, H. Flores-Herrera, N.F.Díaz, A. Molina-Hernández

Histamine in the adult central nervous system acts as a neurotransmitter. This amine is one of the first neurotransmitters to appear during development reaching its maximum concentration simultaneously with peak neuron differentiation. This suggests an important role of histamine in neurogenesis. We have previously shown that histamine is able to increase neuronal differentiation of neural stem cells *in vitro*, by activating histamine type 1 receptor. In this study, we explore the mechanism involved in neuronal differentiation stimulated by histamine. By using two protocols of histamine treatments, one when it is present both during cell proliferation and differentiation, and the other where histamine is only present during cell differentiation.
Immunocytochemistry was performed to evaluating neuron differentiation. To explore the expression of some important transcriptional factors and microRNAs involved in cell commitment, RT-PCR was used. Results indicate that histamine is required during cell proliferation in order to increase neuron differentiation, probably by increasing neuron commitment during this phase. We proposed that the increase in neuron, particularly, in too deep cortical layer cells, commitment by histamine is due to: a) an increase in asymmetric cell division and b) an increase in *Prospero 1* and *Neurogenin 1* expression.

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P2 BEHAVIOURAL ANALYSIS OF H_4 RECEPTOR KNOCKOUT MICE

K. Rossbach , M. Bankstahl, W. Bäumer

Histamine is a well known neurotransmitter that is involved in the regulation of many different brain functions such as waking-sleep cycle, anxiety, cognition, learning and memory as well as basic homeostatic functions. All four histamine receptors ($H_{1\text{-}4}R$) are located in the brain. The expression of the H_4R in the human and rodent brain has only recently been reported and its functional role remains to be elucidated. In this study a first set of behavioural tests was performed with H_4R knockout mice in comparison to age-and sex-matched wild type mice (BALB/c). For a general behavioural screen, a subset of tests from the Irwin screen was performed. General physical characteristics or sensorimotor reflexes showed no abnormalities in the group of H_4R knockout mice. However, H_4R knockout showed an enhanced escape response to approach and struggled more when touched or restrained by tail. For analysing the general activity and anxiety-related behaviour, the open field test, the light and dark box and the elevated plus maze test were performed. Overall the H_4R knockout mice showed only marginal differences in exploratory or anxiety-like behaviour. Furthermore, spatial learning and memory were tested in the Morris water maze test. The H_4R knockout showed a better performance in the Morris water maze test compared to the poor performance of the wildtype mice in this experimental setting. In summary, the H_4R knockout mice seem to react with hyperactivity to handling, but showed no gross differences in other test parameters of the Irwin screen or in their exploratory or anxiety-like behaviour. The improved performance in the Morris water maze test of the H_4R knockout mice may be a first hint for a better learning and memory behaviour of these mice. Thus, the present study reveals that there are

differences in the behavioural phenotype of the H_4R knockout mice, but further studies are required to analyse these differences in more detail.
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P3 DISCOVERY OF NOVEL H1R AND H3R MODULATORS THROUGH A MULTIPLEXED SCREENING STRATEGY UTILIZING SMALL MOLECULES FROM A CNS DOS FOCUSED LIBRARY

E. Holson, J.T. Lowe, S. Kesavan, L.A. Marcaurelle, J. Beaudouin, E. Comer, E. Davoine, K. Dennehy, J.R. Duvall, T. Hanania, M.D. Lee, IV, M. Lewis, D. Lowe, J.-C. Marie, C.A. Mulrooney, G. Muncipinto, T. Petryshen, B-C. Suh, F. Wagner, J. Wei, M.A. Foley

In an effort to synthesize a diverse library of unique compounds biased for CNS drug like properties we utilized *in silico*, *in vitro*, cellular and *in vivo* models to inform library design. An azetidine based core provided a versatile common intermediate en route to a variety of fused, bridged and sprirocyclic ring systems. A phenylethylamine structural motif embedded within the core scaffold provides a pharmacophoric element common to a number of hormones, neurotransmitters, drugs and natural products known to affect the central nervous system. Utilizing an unbiased and multiplexed screening strategy on representative scaffolds we discovered novel, relatively potent and highly selective compounds which modulate the H1 and H3 receptors *in vitro* and *in vivo*. These compounds act as H3 antagonists/inverse agonists and functionally selective H1 agonists and display antidepressant and cognitive enhancing effects in mice. Recent genetic findings implicating histaminergic pathways in the etiology of Tourette syndrome and possible overlap with autism, OCD and ADHD offer intriguing opportunities for therapeutic intervention.

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P4 H_1R SIGNALING IN ANTIGEN PRESENTING CELLS IS DISPENSABLE FOR ELICITING PATHOGENIC T CELLS IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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The histamine H_1 receptor (*Hrh1*/H_1R) was identified as a shared autoimmune disease (SAID) gene in experimental allergic encephalomyelitis (EAE) and autoimmune orchitis, the principal AI models of multiple sclerosis (MS) and idiopathic male infertility, respectively. As a SAID gene, *Hrh1*/H_1R can exert effects in multiple cell types including endothelial cells, T cells, and antigen presenting cells at critical check points during both the induction and effector phases of disease. In this regard, we showed that selective re-expression of H_1R by endothelial cells in *Hrh1*-KO (H_1RKO) mice significantly reduced disease severity whereas H_1R expression by H_1RKO T cells complemented EAE severity and cytokine responses. Given that the H_1R has been reported to influence innate immune cell maturation, differentiation, chemotaxis, and cytokine production, which in turn influences $CD4^+$ T cell effector responses, we selectively re-expressed H_1R in $CD11b^+$ myeloid cells of H_1RKO mice to test the hypothesis that H_1R signaling in these cells contributes to EAE susceptibility and/or T cell effector responses. We demonstrate that transgenic re-expression of H_1R by H_1RKO-$CD11b^+$ cells neither complements EAE susceptibility nor T cell cytokine responses. These results further highlight the cell-specific effects that an AID gene can play in the pathogenesis of complex diseases such as

EAE and MS, and the need for cell-specific targeting in optimizing therapeutic interventions based on such genes.

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P5 INHIBITION OF DEPOLARIZATION-EVOKED [^{3}H]-DOPAMINE RELEASE BY THE ACTIVATION OF THE HUMAN HISTAMINE H_3 RECEPTORS OF 445 AND 365 AMINO ACIDS EXPRESSED IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

G. Nieto-Alamilla, J. Escamilla-Sánchez, R. González-Pantoja, J.-A. Arias-Montaño

The histamine H_3 receptor (H_3R) is mainly located on nerve terminals and controls the release of histamine and other transmitters, including dopamine. The alternative splicing of the human H_3R gene produces several receptor isoforms, of which the variants of 365 and 445 amino acids (hH_3R_{365} and hH_3R_{445}) are expressed in the brain. The objective of this work was thus to determine, by using a cellular model, whether these two isoforms regulate in a different manner neurotransmitter release.
Two sub-lines of human neuroblastoma SH-SY5Y cells stably expressing either isoform (hH_3R_{365} or hH_3R_{445}) were generated with expression levels of 583 ± 151 and 805 ± 175 fmol/mg protein and *K*d values for [^{3}H]-*N*-methyl-histamine of 0.90 ± 0.13 and 0.86 ± 0.12 nM, respectively. There were no significant differences in the affinities of both isoforms for the H_3R ligands histamine (p*K*i values 8.50 ± 0.10 and 8.55 ± 0.07), R-α-methylhistamine (RAMH, 9.45 ± 0.06 and 9.63 ± 0.06) or clobenpropit (8.70 ± 0.09 and 8.95 ± 0.12).
The H_3R agonist RAMH was more efficacious to inhibit forskolin-induced cAMP accumulation in the SH-SY5Y-hH_3R_{445} cell line (-70.6 ± 5.2% versus -38.4 ± 5.4% for the SH-SY5Y-hH_3R_{365} cells), without significant difference in the pIC_{50} estimates (8.77 ± 0.17 versus 8.42 ± 0.14).
Neurotransmitter release was assayed in cells differentiated by treatment for 7 days with 12-*O*-tetradecanoylphorbol-13-acetate, TPA (20 nM), and retinoic acid (10 μM). The release of [^{3}H]-dopamine induced by depolarization with high K^+ (100 mM) was significantly reduced by RAMH (100 nM), but the effect was significantly larger in the SH-SY5Y-hH_3R_{445} cell line (-30.9 ± 4.2%) than in the cells expressing the hH_3R_{365} isoform (-15.1 ± 4.8%).
Our results indicate that in accord with previous data for other signaling pathways, the hH_3R_{445} isoform is more efficacious in inhibiting depolarization-evoked neurotransmitter release.
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P6 THE HISTAMINE H_3 RECEPTOR ANTAGONIST A-960656 IS EFFECTIVE IN ANIMAL MODELS OF OSTEOARTHRITIS AND NEUROPATHIC PAIN

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Histamine H_3 receptor antagonists have been widely reported to improve performance in preclinical models of cognition and efficacy in pain models has recently been described. A-960656 was profiled as a new structural chemotype. A-960656 was potent *in vitro* in histamine H_3 receptor binding assays (rat K_i=76 nM, human K_i=21 nM), exhibited functional antagonism in blocking agonist-induced [^{35}S] GTPγS binding (rat H_3 K_b=107 nM, human H_3 K_b=22 nM), and was highly specific for H_3 receptors in broad screens for non-H_3R sites. In a spinal nerve ligation model of neuropathic pain in rat, oral

doses of 1 and 3 mg/kg were effective 1 hour post dosing with an ED_{50} of 2.1 mg/kg and a blood EC_{50} of 639 ng/ml. In a model of osteoarthritis pain, oral doses of 0.1, 0.3, and 1 mg/kg were effective 1 hour post dosing with an ED_{50} of 0.52mg/kg and a blood EC_{50} of 233 ng/ml. The antinociceptive effect of A-960656 in both pain models was maintained after sub-chronic dosing up to 12 days. A-960656 had excellent rat pharmacokinetics ($t_{1/2}$=1.9 h, 84% oral bioavailability) with rapid and efficient brain penetration, and was well tolerated in CNS behavioral safety screens. A-960656 has properties well suited to probe the pharmacology of histamine H_3 receptors in pain. Its potency and efficacy in animal pain models provides support to the notion that histamine H_3 receptor antagonists can effectively attenuate nociceptive processes.

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P7 THE NOVEL HISTAMINE H_3 RECEPTOR ANTAGONIST ST-1283 ATTENUATES ETHANOL CONSUMPTION AND PREFERENCE IN MICE

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Growing evidence supports a role for the central histaminergic system in its modulatory influence on drug addiction in general and alcohol-use disorders in particular. Therefore, a new class of tricyclic nonimidazole-based histamine H_3 receptor (H_3R) antagonists has been developed and investigated on their ethanol consumption and preference effects in mice as a model of drug addiction. The compound **ST-1283** is a parent ligand for this new class and can be seen as ether product of the robust H_3R pharmacacophore, 1-(3-chloropropyl)piperidine and 4-(3-methyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-yl)phenol.

Here **ST-1283** has been investigated on *in vitro* and *in vivo* studies. In addition to binding studies at H_3R, the effects on ethanol consumption and preference in mice have been studied as a model of drug addiction. Oral ethanol, saccharin and quinine intake was assessed in a two-bottle choice paradigm using escalating concentrations of alcohol or tasting solutions. In these screenings, 2.5, 5, 10 and 20% alcohol, 0.04% and 0.08% saccharin, and 0.02 mM and 0.04 mM quinine solutions have been used.

Compound **ST-1283** is a high affinity ligand at human H_3R with subnanomolar affinity (pK_i value of 9.62). Administration of either vehicle or **ST-1283** (2.5, 5 and 10 mg/kg, i.p.) dose-dependently and significantly decreased alcohol consumption and preference without affecting total fluid intake. More interestingly, vehicle and **ST-1283** (5 mg/kg) treated mice showed similar consumption and preference to increasing concentration of both sweet and bitter tastes. Our results show that **ST-1283** may decrease voluntary ethanol consumption in male mice by altering its reinforcing effects. The lack of significant effect on quinine and saccharin consumption suggests that **ST-1283** does not simply decrease consumption of all fluids. These findings provide further evidence for the role of central H_3R in the mediation of voluntary ethanol consumption in mice and may offer further perspectives in the pharmacotherapy of ethanol addiction.

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P8 HISTAMINE PROMOTES NEURONAL DIFFERENTIATION OF CULTURED MIDBRAIN NEURAL PRECURSORS, BUT DIMINISHES DOPAMINE NEURON NUMBERS *IN VITRO* AND *IN VIVO*

I. Escobedo, A. Molina-Hernández, I. Velasco

During embryonic development, histamine (HA) is one of the first neurotransmitters to appear, presenting higher concentrations than those observed in the adult central nervous system. Particularly, in the region of ventral midbrain (VM), HA increases its concentration up to 5-fold at embryonic day 14-16 and then it decreases until birth, reaching the low concentrations that are present in the adult brain. Neuronal differentiation in the VM correlates temporally with the increase in the concentration of HA in this region. The role of HA during brain development has been elusive. In this work, we aimed to study the correlation between HA increases with the process of neurogenesis on rat VM *in vitro* and *in vivo*. We analyzed the effect of several HA concentrations during proliferation, differentiation and cell death of VM neural precursor cells (NPC) *in vitro.* We found that VM cultures express HA receptors, and that HA was able to significantly increase neuronal differentiation from 21% to 37%. Pharmacological assays revealed that the effect of HA on neuronal differentiation is mainly due to activation of H_2 receptors. We observed that HA increased 2-fold apoptotic cell death compared to control conditions, evaluated by TUNEL assay. An interesting finding was that the proportion of dopaminergic neurons was significantly decreased after HA treatment of cultured VM NPC. We also evaluated the role of HA *in vivo* through intrauterine injections in the developing brain, and found that HA administration decreased the number of dopaminergic neurons in the VM, consistent with the data obtained *in vitro*. Taken together, these results suggest that HA might be acting as a transient developmental signal that modulates neurogenesis and dopamine neuron differentiation/survival in the midbrain.

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P9 INVOLVEMENT OF THE CENTRAL HISTAMINERGIC SYSTEM IN THYROTROPIN RELEASING HORMONE-INDUCED RESUSCITATING EFFECT IN HAEMORRHAGIC SHOCK IN RATS

J. Jochem

Thyrotropin releasing hormone (TRH) is mainly secreted by neurons of the paraventricular nucleus of the hypothalamus. Apart from the role in the hypothalamus-pituitary-thyroid axis, it influences many functions of the central nervous system, including the regulation of feeding and locomotor behaviours, pain perception and respiratory and cardiovascular functions. Intravenously (iv) administered TRH evokes the resuscitating effect in haemorrhage-shocked rats, with increases in mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). The mechanism of the effect is associated with the activation of brain cholinergic neurons and central muscarinic receptors. Interestingly, recent *in vitro* studies by the group of Haas and Sergeeva demonstrate the excitation of histaminergic neurons of the tuberomamillary nucleus (TMN) by TRH. Since we revealed previously the resuscitating effect resulting from the central histaminergic system activation, and immunohistochemical analysis showed TRH type 2 receptors within TMN neurons, the aim of the present study was to examine an involvement of the histaminergic system in TRH-evoked effects in haemorrhagic shock. Experiments were carried out in ketamine/xylazine-anaesthetised male Wistar rats subjected to haemorrhagic hypotension, with MAP stabilized at 20-25 mmHg, which resulted in the death of all control animals within 30 min. TRH (3 mg/kg) given iv at 5 min of critical hypotension evoked long-lasting increases in MAP, PP and HR, with a 100% survival at 2 h. The effect was inhibited by intracerebroventricular pre-treatment with the histamine H_1 receptor antagonist chlorpheniramine (50 nmol), but not with ranitidine (50 nmol), clobenpropit (42.5 nmol) and JNJ 10191584 (25 nmol) – H_2, H_3 and H_4 receptor blockers, respectively. In conclusion, the results of the

present study demonstrate for the first time the involvement of the histaminergic system in TRH-induced resuscitating effect in haemorrhagic shock in rats.

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P10 INTRAVENOUS INFUSION OF ASCORBIC ACID REDUCES PLASMA HISTAMINE LEVELS

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Histamine (HA) represents a mediator which plays amongst others an important role in allergic and inflammatory diseases as well as HA intolerance. Degradation of HA in humans occurs through Histamine-N-Methyltransferase (HNMT) and Diaminooxidase (DAO).
Ascorbic acid represents an essential vitamin and an antioxidant agent. Initially in guinea pigs and later in test humans, an inverse relationship between plasma HA and plasma ascorbic acid concentrations was found.
90 test persons were treated with 7.5g ascorbic acid (Pascoe, Giessen) intravenously over 60 minutes. Directly before and one hour after infusion plasma HA levels and DAO-activity were measured using an ELISA technique (IBL, Hamburg and Sciotech, Tulln).
Plasma HA concentrations were distributed over a range from 0.15 – 0.75ng/ml. A statistical significant decline was sown in all test persons of 34.9% after ascorbic acid infusion ($p<0.00005$). After separating the test individuals into a subgroup with and without allergic diseases, both groups showed a significant decline in HA concentration (no allergy: 30.4%; allergics: 47.9%). Interestingly, individuals with allergic diseases had a significantly greater impact on histamine concentrations ($p=0.021$) than controls. However, DAO levels did not show any difference before and after ascorbic acid infusion (127.74 HDU/ml vs 110.91 HDU/ml, $p=0.385$).
This study demonstrated a direct impact of ascorbic acid administration on plasma HA levels. Ascorbic acid reduced plasma HA levels significantly in allergics and non-allergic patients. Patients with allergic diseases showed a statistically greater decline in histamine levels. Thus, we are able to confirm previous results found in guinea pigs and to give some objective data for ascorbic acid infusion in the treatment of HA intolerance symptoms, mastocytosis, polyvalent allergics, inflammatory disease states etc for the future. Since no change in DAO activity was found, the mechanisms behind the beneficial action of ascorbic acid remain to be further explored.

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P11 EXOGENOUS SALSOLINOL ACTS ON MAST CELLS AND INTERSTITIAL CELLS OF CAJAL IN THE RAT JEJUNUM

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Mast cells (MC) and the interstitial cells of Cajal (ICC, pacemaker cells) develop close spatial contact in the gastrointestinal (GI) tract. Intestinal motility disturbances are frequently associated with a reduced number of ICC as well as with immune cells. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is an endogenous agent primally formed by non-enzymatic pathway from aromatic amines precursors leading to neurotoxic N-methyltetrahydroquinolinium ions that may play a role in the etiology of Parkinson's disease (PD). Gastrointestinal dysmotility observed in PD

patients is suggested to be partly associated with the peripheral action of salsolinol. The aim of this study was to evaluate the chronic effects of salsolinol on MC and ICC in the jejunum of rats.
Male Wistar rats were subjected to continuous intraperitoneal administration of salsolinol (50 mg in total per animal) with ALZET (Durtec, USA) osmotic mini-pumps for two weeks (delivery rate 0.5 µL/h, n=4, Salsolinol 1) or four weeks (delivery rate 0.25 µL/h, n=4, Salsolinol 2). An equivalent group of rats (n=8) served as the control group. At the end of the experiment animals were decapitated and fragments of jejunum were removed. Longitudinal muscle–myenteric plexus preparations (LMMP) were double-stained with anti-c-Kit polyclonal antibody (Dako, USA) and anti-tryptase polyclonal antibody (Dako, USA).
The number of cells double-stained with anti-c-Kit and anti-tryptase (i.e. MC) in the GI wall was decreased in both salsolinol-treated groups compared to control group. However, the number of degranulated MC was elevated in both salsolinol-treated groups compared to control group. The number of cells stained with anti-c-Kit (i.e. ICC) was lower in both salsolinol-treated groups in comparison with the control group.
In our model, chronic administration of salsolinol exerts destructive influence over MC and ICC, and thus may lead to a GI impairment.

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P12 ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR HISTAMINE AND METHYLHISTAMINE DETERMINATION IN TISSUES AND PLASMA

S. Rajtar Osredkar

So far we have been able to measure histamine level in many different ways, including capillary electrochromatography, radioenzymatic assay, enzyme immunoassay, gas chromatography, spectrofluorometry and high performance liquid chromatography (HPLC) and others. Most of these techniques, however, suffer from some disadvantages, such as low specificity and sensitivity, possible appearance of interfering substances, time consuming sample preparation or expensive instrumentation. Histamine concentrations that provoke life threatening systemic effects are very low, often just above the detection limit of the above mentioned methods. We are presenting here an improved method for measuring histamine and methylhistamine at ng/ml concentrations in biological samples. We will describe the set-up of the ultra high performance liquid chromatography (U-HPLC) assay for histamine and methylhistamine measurement in guinea pig plasma and tissues. We determined the sensitivity, accuracy, reproducibility, and detection limit of U-HPLC. The performance of U-HPLC on a column with a 2.6 µm core-shell particle was also compared with HPLC on a column with standard particle size (5 µm). After optimising pressure, mobile phase and concentration of derivatisation agent, we attained the capability of detecting very low histamine and methylhistamine concentration, even as low as 2 ng/ml. To reach this detection limit, deproteinization, ion exchange chromatography procedures as well as extraction with acetonitrile and methanol was necessary. Histamine and methylhistamine retention times were 4.1 and 6.4 min, respectively, which is significantly faster than on column with standard particle size (retention times 12 and 16 min, respectively). The most important advantages of U-HPLC are high reproducibility and low detection limit, however, sample preparation is time consuming and needs further improvement.

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P13 DIAMINE OXIDASE IS PRESENT IN SEMINAL PLASMA FROM MAN BUT NOT IN THAT FROM OTHER MAMMALS

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Human seminal plasma contains considerable amounts of diamine oxidase (DAO). However, information on the function of human seminal plasma DAO, on DAO in seminal plasma from other mammals and on the presence of the second histamine inactivating enzyme, histamine N-methyltransferase (HMT) in sperm was scarce. Therefore, we set out to investigate the presence of DAO and HMT in ejaculates from different mammalian species.
Semen samples were collected from pig, cattle, horse, dog and humans and seminal plasma and sperm cells were separated by centrifugation. DAO and HMT activities were determined in seminal plasma from the pre-, main and post-ejaculate and in homogenates of sperm cells using radiometric assays. DAO protein was analyzed in parallel samples by Western blotting with specific antibodies. Total mRNA was prepared from sperm cells and the expression of DAO and HMT was analyzed by RT-PCR using specific primers.
DAO activity was measurable only in human seminal plasma but not in seminal plasma from the other species tested nor in sperm cell homogenates. The presence of DAO in human seminal plasma and its absence in the other samples was confirmed by Western blotting with DAO specific antibodies. HMT activity was not detectable in any of the samples analyzed. Neither DAO mRNA nor HMT mRNA was detectable in total sperm cell RNA from any of the species.
From these results we conclude that DAO is present in human seminal plasma but not in seminal plasma from other mammals nor in sperm cells. Human seminal plasma DAO likely originates from the prostate gland or the testis where DAO expression is detectable at the mRNA level. HMT appears not to be present in mammalian sperm. The presence of DAO only in human seminal plasma is an interesting finding whose functional importance in the fertilization process with respect to histamine and possibly polyamine inactivation remains to be determined.

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P14 CHARACTERIZATION OF THE EP RECEPTOR EXPRESSED BY HUMAN LUNG MAST CELLS USING NOVEL EP RECEPTOR ANTAGONISTS

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Prostaglandin E_2 (PGE_2) inhibits histamine release from human lung mast cells (HLMC). PGE_2 mediates its effects through EP receptors, four of which have been identified. Our previous studies suggest that PGE_2 acts through EP_2 receptors in HLMC (Kay *et al.,* 2006). The recent development of more potent and selective EP_2 and EP_4 antagonists (Murase *et al*., 2008; af Forselles *et al*., 2011) has allowed us to reappraise EP receptor expression in HLMC.

HLMC were generated by physical and enzymatic disruption of lung tissue. Cells were incubated with or without antagonist (50 min), then agonist (10 min) before challenge with anti-IgE (25 min). Histamine released into the supernatants was assayed using an automated fluorometric technique.

To determine cyclic-AMP levels, purified HLMC were incubated (10 min) with agonist, the reaction terminated using ice-cold ethanol and cyclic-AMP measured using commercially available EIA kits.

PGE_2 inhibited IgE-mediated histamine release in a concentration dependent manner (pD_2, 5.8±0.1). The EP_2-selective agonist butaprost also inhibited histamine release whereas neither sulprostone ($EP_{1/3}$ agonist), nor 17-phenyl-trinor-PGE_2 (EP_1 agonist) had any effect. PGE_2 increased total cyclic-AMP levels in HLMC as did butaprost. Neither sulprostone nor 17-phenyl-trinor-PGE_2 was effective at increasing cyclic-AMP. Overall these data with agonists suggest that PGE_2 acts on HLMC through EP_2 or EP_4 receptors. The effects of selective EP_2 (PF-04418948) and EP_4 (CJ-042794) antagonists on the inhibition of histamine release by PGE_2 were studied. The EP_2 antagonist PF-04418948 (pK_B, 6.78) reversed the inhibition of histamine release by PGE_2. By contrast, CJ-042794 had no effect on the inhibitory effects of PGE_2.

The use of novel EP selective antagonists confirms that PGE_2 acts through EP_2 receptors to stabilize HLMC.

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P15 EFFECT OF PKC ON HMC-1^{560} AND HMC-$1^{560,816}$ MAST CELL LINES ACTIVATION

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Several pathways are related with mast cell activation, such as calcium (Ca^{2+}), Protein Kinase A (PKA), Protein Kinase C (PKC) and phosphatases. HMC-1 (Human Mast Cell) is a human mast cell line which has in their membrane the c-kit receptor of tyrosine kinase (TyrK). The natural ligand of c-kit is the stem cell factor, however, the proliferation of this cell line is independent of this factor. The two mast cell lines, HMC-1^{560} and HMC-$1^{560,816}$, differ in one mutation in the c-kit proto-oncogene. Both sublines have the Gly-560-Val mutation but HMC-$1^{560,816}$ cell line carries another mutation, Asp-816-Val in the intracellular side of c-kit which modifies the conformation of TyrK. Since PKC is an important mediator of mast cell exocytosis, the effect of PKC activation was studied in both mast cell lines. PKC activation increases (HMC-1^{560}) or inhibits (HMC-$1^{560,816}$) mast cell exocytosis depending on cellular model. STI571 (imatinib mesylate) is a tyrosine kinase inhibitor and thus inhibits kinase activity of c-kit. Is widely known that STI571 failed to inhibit HMC-$1^{560,816}$ cell line proliferation, whereas it is effective in HMC-1^{560} mast cell line. The effect of STI571 and PKC activation was analyzed in this work. An opposite STI571 effect on PKC expression was observed in HMC-1^{560} and HMC-$1^{560,816}$ cell lines. Thus, STI571 increases classical PKCs expression in HMC-$1^{560,816}$, nevertheless STI571 does not have this effect in HMC-1^{560}. Besides, STI571 effect described in HMC-$1^{560,816}$ is blocked when PKC is activated. Moreover, cytosolic expression of PKCδ, an isoform related with apoptosis, was studied in both cell lines. STI571 decreases cytosolic PKCδ expression in HMC-1^{560} while the compound does not affect protein expression in HMC-$1^{560,816}$. Finally, STI571 treatment induces PKCδ translocation to the nucleus in HMC-1^{560} cells, whereas in HMC-$1^{560,816}$ cell line, this translocation does not occur.

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P16 NOVEL MAST CELL-STABILISING AMINE DERIVATIVES OF 3,4 DIHYDRONAPHTHALEN-1(2H)-ONE AND 6,7,8,9-TETRAHYDRO-5H-BENZO[7]ANNULEN-5-ONE

J.W. Barlow, T. Zhang, O. Woods, J.J. Walsh

Mast cells are densely granulated cells, traditionally associated with the pathogenesis of allergic reactions; however, their additional roles are increasingly being recognised. As examples, mast cells are involved in cell-mediated immune reactions, are a component of the host reaction to infection, and have functions in tumour angiogenesis and tissue repair. The aim of this work was to conduct an assessment of the potential anti-allergic activity of a novel series of cyclic amino derivatives of tetralone and benzosuberone. The primary steps in their synthesis involved a Wohl-Ziegler bromination step followed by a substitution reaction with the appropriate amine. Alkylation of the secondary amines thus formed was effected with a series of alkylhalides to afford the title compounds for evaluation. Percoll-purified rat peritoneal mast cells were used for the *in vitro* studies which employed compound 48/80 and calcium ionophore A231867 to induce degranulation. The *in vivo* passive cutaneous anaphylaxis assay was used to evaluate the most active compounds from the *in vitro* work. *In vitro* investigation of the mast cell stabilising activity revealed that optimal activity appeared to reside in a tertiary amine bearing either; parent bicyclic system, an unsaturated cyclohexene, and thirdly, a benzyl or substituted benzyl motif. It further appears that for *in vivo* activity the unsaturated alicyclic system on nitrogen is critical. This suggests that while ring expansion of the hydroaromatic core is permissible without loss of activity *in vivo*, there must be an unsaturated alicyclic component.

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P17 HIGH AFFINITY DIMERIC H_3R LIGANDS INDICATE RECEPTOR DIMERS/OLIGOMERS

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Despite recent progress in clinical trials with hH$_3$R inverse agonists/antagonists, the molecular complexity of H_3R signaling and organization leaves many open questions, e.g. the impact of receptor (homo)dimerization/oligomerization. Based on the recently published crystallographic X-ray structure of the monomeric hH$_1$R[1] we have developed a homology model of the hH$_3$R which has then be utilised to build up a hH$_3$R homodimer model taking advantage of the crystallographic X-ray structure of dimeric CXCR4.[2] This model has been the basis in the search for the optimal linker length for divalent hH$_3$R ligands which stabilize dimeric receptors by lipophilic bridging the pharmacophoric elements within the transmembrane regions. The Asp114 binding pockets have been taken as basic orientation for the measurement of a distance of about 40 Å in pharmacophoric elements. Taking into account some flexibility in the linking chain with different lipophilic transmembrane interactions a spacer of six to 16 methylene units may be acceptable in this model.
Different derivatives of the antagonist JNJ-5207852 have been prepared with different linking elements as we have shown in previous studies that numerous structural variations can be performed maintaining high hH$_3$R affinity.[3,4] The monomeric precursors have been linked to dimeric compounds by peptide like amide bonds or by triazole elements conveniently obtained by click chemistry in a tail-to-tail or tail-to-head manner. This allowed a certain degree of freedom on the recognition of different pharmacophoric warheads since the exact binding mode is not clarified. Most of the di- and structurally related monovalent compounds displayed, with a displacement assay, binding affinities in a comparable nanomolar concentration range, but displayed, with strong differences in Hill slope, a different mode/ratio in receptor binding.

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P18 INTERACTION OF N-METHYL PIPERAZINE DERIVATIVES WITH PTEN PROMOTER

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The aim of this study was the exploration of the interaction of N-methylpiperazine derivatives with the promoter of *PTEN* (**p**hosphatase and **ten**sin homolog deleted on chromosome **ten**). *PTEN* is one of the most frequently mutated tumor suppressor genes in human cancer, with a frequency approaching that of p53. The protein encoded by *PTEN* is a tyrosine phosphatase. Although it can dephosphorylate proteins, its primary targets are highly specialized membrane lipids, mainly facilitating the removal of the phosphate group from the inositol rings. The examined compounds were synthesized with histamine H_4R targeting activity.
PTEN promoter was amplified together with exon-1 using human genomic DNA that was extracted from blood. Each compound, at a final concentration of 2 mM, was incubated with an equal amount of PCR product at 24 ^{o}C for 30 min. Subsequently the mixtures were electrophoresed on 2% w/v agarose gel.
TR-33 reduced the motility of the PCR product to a greater extent than TR-18 and TR-19. We conclude, that in the group of triazines the substitution of the 3-pyridyl in TR-33 with the 4-bromophenyl in TR-18 reduced the activity. The reduction was even greater when the 2-naphthylmethyl was introduced in TR-19. No effect was observed with TR-20 and TR-28 that differ also with aryl substituent from TR-33. Comparing KB-1, KB-3, KB-8 and KB-28.2 the best result was observed with KB-1 followed by KB-3 and KB-8, while KB-28.2 had no effect. It is interesting to notice that KB-1 and TR-33 possess similar structures with aryl substituent directly connected with triazine core. The best results were obtained among amides with CJ-7, L-58 and L-11. CJ-16, CJ-22 and L-60 had no effect.

In conclusion, some of the N-methylpiperazine derivatives influenced the motility of *PTEN* promoter. This implied an interaction with the promoter that could regulate *PTEN* expression. A favourable SAR for this effect is suggested.

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P19 THE HISTAMINE H_4 RECEPTOR IS OVEREXPRESSED IN THE KIDNEY OF DIABETIC RATS

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Histamine is known to be synthesized in the glomeruli, to increase second messenger levels in isolated glomeruli and to influence renal hemodynamics, including microcirculation. Previous studies

demonstrated the glomerular expression of histamine H_1 receptors (H_1R) and H_2R, while the most recently discovered H_3R and H_4R were poorly investigated. The aim of this study was to evaluate the H_4R expression in the rat kidney. As renal histamine concentration was found to be significantly increased in streptozotocin (STZ)-treated rats, a further aim was to evaluate whether the kidney H_4R density varies in diabetic rats. Insulinopenic diabetes was induced in 12 out of 24 8-week-old male Wistar rats by a single i.v. injection of STZ, and animals were sacrificed 6 weeks later. Serum and urinary evaluation was performed to assess glycaemia, creatinine clearance and proteinuria. The kidneys were collected and processed for PCR or immunohistochemistry analyses. Diabetic rats (glycaemia >300 mg/dl) had a higher creatinine clearance and proteinuria than nondiabetic animals. The H_4R mRNA expression in control rats was at a very low level. Consistently, H_4R was undetectable by immunohistochemistry using a well validated anti-H_4R antibody. In contrast, in diabetic rats H_4R was profoundly ***upregulated***.
Immunohistochemistry experiments revealed a higher immune-positivity in the cortex and medulla in comparison to the papilla. To ascertain the cellular localization of H_4R, colocalization experiments were performed. When H_4R immunostaining was compared to that of Tamm-Horsfall glycoprotein, a close overlap in expression topology was observed.
In conclusion, our results indicated that H_4R is expressed in the kidney of healthy rats, although at a very low level, and it is upregulated in diabetic male rats. This receptor is expressed by resident renal cells of the thick ascending limb of the loop of Henlé, thus suggesting a possible role in modulating the transmembrane soluble transport processes.

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P20 H_1 VERSUS H_4 ANTI-HISTAMINES IN HUMAN NEUTROPHIL OXIDATIVE BURST

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Professional phagocytes play a crucial role in phagocytosis, immune reactions and pathological processes, including inflammation. Histamine possesses a regulatory role in these reactions.
We found that H_1R anti-histamines (AH) inhibited the oxidative burst of professional phagocytes according to their chemical structure and physico-chemical properties. In this study, we compared the effect of H_1R and H_4R AH on the oxidative burst of human blood and isolated neutrophils by means of luminol and/or isoluminol enhanced chemiluminescence (CL) in vitro. Thirteen compounds of the 1st and 2nd H_1R AH generation, and JNJ7777120, JNJ10191584 and thioperamine, H_4R AH were analysed.
Most of the H_1R AH tested dose-dependently inhibited stimulated CL produced by neutrophils. Histamine was effective only at the extracellular level. H_1R AH inhibited CL both at extracellular (scavenging) and intracellular level of neutrophils (suggestive of interference with regulatory enzymes). It is expected that the inhibition is a result of nonreceptor rather than receptor interaction. The H_4R AH JNJ7777120 and JNJ10191584 tested showed very weak inhibition on whole blood and extracellular CL, without any dose-response relationship and they were not active intracellularly. Thioperamide potentiated CL both in whole blood and in isolated neutrophils.
The difference between H_1R and H_4R AH effect on oxidative burst requires further analysis at the molecular level and through structure-activity relationship. In comparison with H_1R AH, the H_4R

compounds tested displayed a very low scavenging and intracellular antioxidative and oxidative burst suppressing activity.

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P21 EFFECTS OF HISTAMINE H_4 RECEPTOR AGONISTS AND ANTAGONISTS ON CARRAGEENAN-INDUCED INFLAMMATION IN RATS

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The paradigm that the histamine H_4 receptor (H_4R) blockade is related to inhibition of inflammation has been challenged by the recently reported agonist activity of JNJ7777120, the "standard" H_4R antagonist, in some settings (Neumann et al., 2010; Rosethorne et al., 2011; Seifert et al., 2011). This confirms previous observations from our lab (Coruzzi & Adami, 2009; Adami et al., 2010), which showed anti-inflammatory effects of the H_4R agonists VUF8430 and VUF10460 on carrageenan-induced paw edema in the rat.
In the present study, several H_4R ligands were characterized for their H_4R activity (agonism, partial agonism, neutral antagonism) at rat GTPγS binding assay and tested *in vivo* against the carrageenan-induced paw edema in the rat. Among the compounds tested, VUF8430 behaved as a full agonist (α=0.92) in GTPγS assay and induced at 100 mg/kg subcutaneously (sc), a 92% inhibition of paw edema; JNJ7777120, VUF10460 and clobenpropit behaved as partial agonists (α=0.42, 0.13 and 0.21, respectively) and induced 77.02%, 65.58% and 66.73% inhibition of paw edema, respectively. VUF10519 and VUF10497 were neutral antagonists at GTPγS assay (α=0) and induced 38.18% and 32.36% of paw edema, respectively.
In conclusion, the present data suggest that the functional activity of H_4R ligands in the rat assay *in vivo* do not correlate with the functional behaviour *in vitro*. Surprisingly, the most effective inhibition of carrageenan-induced edema was obtained with H_4R agonists rather with H_4R antagonists.

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P22 COMBINATION OF PROTOTYPICAL HISTAMINE H_3 AND H_4 RECEPTOR PHARMACOPHORES

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The human histamine H_3 and H_4 receptor subtypes (hH$_3$R, hH$_4$R) are structurally highly related G protein-coupled receptors. Preclinical and clinical data gave hints at an involvement of the two receptors in different diseases related to itch and pain.

According to the structural requirements responsible for affinity and selectivity for both receptor subtypes, we investigated the combination of 2-aminopyrimidines and related structures (for H_4R) with the well-established and robust (3-piperidinopropoxy)phenyl pharmacophore (for H_3R). In an effort to design new structurally hybrid compounds, a novel series of substituted pyrimidines were synthesized.
The compounds were prepared by microwave-assisted aromatic nucleophilic substitution reactions of either 2-amino-4,5-dichloropyrimidine or 2,4-dichloropyrimidine. The *in vitro* receptor binding properties were obtained by radioligand displacement assay in HEK-293 cells stably expressing the *h*H_3R and on Sf9 cells transiently expressing the *h*H_4R (co-expressed with $G\alpha i2$ and $G\beta_1\gamma_2$ subunits).
Slight structural changes of the *h*H_4R scaffold evoked severe changes in *h*H_4R affinity, although the 2,4-diaminopyrimidine scaffold can be found in many potent *h*H_4R ligands. In contrast, *h*H_3R affinity was barely affected by introducing the pyrimidine-related motif in the H_3R pharmacophore. Novel compounds with *h*H_3R binding properties in the nanomolar concentration range have been achieved.

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P23 DERIVATIVES OF 2-AMINO-1,3,5-TRIAZINE AS NEW HISTAMINE H_4 RECEPTOR LIGANDS

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Histamine - the biogenic monoamine - fulfills important physiological functions through four histamine receptor subtypes (H_1-H_4R). The youngest member of this receptor family is the H_4 receptor (H_4R) which was discovered at the turn of 21st century independently by several research groups [1]. Because of H_4R expression mainly in cells and tissues of immune system (monocytes, mast cells, dendritic cells, eosinophils, basophils, T-leukocytes) its role in inflammatory and (auto)immunological processes and disorders was suggested. Positive effects were observed in treatment of animal models of some diseases (e.g. allergic rhinitis, asthma, pruritus or pain) in the presence of H_4R antagonists/inverse agonists [2,3].
Extending our SAR investigation in the group of 1,3,5-triazine derivatives - based on previous results and literature data [4,5] we searched for new potent and selective H_4R ligands in the group of (4-methylpiperazin-1-yl)-1,3,5-triazin-2-amines by changing aryl substituent in 6-position to heteroaryl ring system, e.g.(un)substituted thiophene, benzothiophene, pyridine or imidazopyridine.
The compounds were obtained by the direct reaction of appropriate carboxylic esters with guanidine derivative. *In silico* predictions of toxicity and drug-likeness by newly obtained compounds were also carried out. Compounds were evaluated for their affinity at H_4R with radioligand binding assays on transiently expressing recombinant human H_4R in the *Sf9* insect cells using [^{3}H] histamine as radioligand. As one of the most promising substitution pattern the thienyl derivatives were characterized with significant affinity for H_4 receptor, e.g. 4-(3-chlorothienyl-2-yl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine showed a K_i value of 137 nM.

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P24 HISTAMINE AND CLOZAPINE TREATMENTS INHIBIT TUMOR GROWTH AND INCREASE MEDIAN SURVIVAL IN HUMAN MELANOMA XENOGRAFT MODEL

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Melanoma accounts for less than 5% of skin cancer cases but causes a large majority of skin cancer deaths. The expression of all histamine receptors (HR) subtypes were demonstrated in human melanoma cell lines, and the activation of the H_4R in WM35 primary and M1/15 highly metastatic human melanoma cells inhibits proliferation and migration, and induces differentiation and senescence. The aim of this work was to evaluate the *in vivo* antitumor potential of histamine (HA) and clozapine (CLZ, H_4R agonist) on human melanoma. An experimental model was developed by subcutaneous (sc) injection of M1/15 cells into the right flank of athymic nude mice. Animals were separated into three groups: control, HA (1mg.kg^{-1}, sc) and CLZ (1mg.kg^{-1}, sc). Tumors were excised and the expression levels of H_4R, HA and histidine decarboxilase (HDC) were studied by immunohistochemistry. Cell growth was assessed by PCNA expression and by mitotic index (MI). Finally, vascularization was determined by Massons trichromic staining, and invasion markers by metalloproteases (MMP-2 and MMP-9) expression levels. Mice receiving HA or CLZ showed a median survival increase (61 d) vs. control group (40 d) (treated with saline solution) ($p<0.05$). Tumor volumes after 70 days of HA (2.7 cm^3) or CLZ (3.0 cm^3) treatment were significantly lower than in control mice (12.8 cm^3) ($p<0.01$). Treatment with HA (12.8 ± 4.3 d) or CLZ (13.8 ± 4.3 d) increases tumor doubling time vs. control group (5.0 ± 1.1 d), $p<0.05$. PCNA, MI, intratumoral neovascularization and the expression of MMP-2 and MMP-9 were diminished in HA and in CLZ treated mice, while the expression of H_4R, HA and HDC seemed not to be modified. We conclude that HA and CLZ show an antitumor effect in human melanoma. Further studies are needed to corroborate the H_4R importance as potential target for new drug development for the treatment of this disease.

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P25 H_3 AND $H_3/_4$ ANTAGONISTS MODULATE CYTOKINE SYNTHESIS IN PATIENTS WITH ACUTE SENSORINEURAL HEARING LOSS WITHDRAWN

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Acute sensorineural hearing loss (ONT) is one of the most common diseases and urgent problems of ENT. Proposed theories of causation include viral infections, vascular occlusion and immune system-mediated mechanisms. During the last few years, it have been shown that one of the effective medications for the ONT treatment is Betahistine (BH) which possess a very strong affinity as an antagonist for histamine H_3 receptors and due to this effect increases the levels of neurotransmitters released from the nerve endings. At the same time BH exerts a weak affinity for

histamine H_1 receptors as an agonist. The goal of the study was to investigate the modulation of immune responses in ONT patients treated with BH, as well as to study the cytokine production by mononuclear cells (PBMC) pretreated by $H_{3/4}$ antagonist, imoproxifan-IMP (10^{-5}M/ml). The concentration of pro- and anti-inflammatory cytokines (IL1$_\beta$, TNFα, IL4 and INFγ) have been assessed by ELISA method in sera of patients treated by BH and in supernatant of PBMC cultivated in the presence of BH or IMP. It has been shown that IMP as well as BH decrease IL1$_\beta$, TNFα and INFγ synthesis and this effect was more pronounced for cells treated by IMP. The decrease in cytokine production was higher on the 14th day of treatment in comparison to non-treated patients (day 1 of the disease). Both histamine antagonists increase IL4 synthesis by PBMC of ONT patients. It was determined that IMP and BH induced more pronounced effects on the cytokine production in the group of effectively treated patients versus the group of patients with low response to the treatment. Thus, H_3R, as well as $H_{3/4}$R, antagonists influence on pro-and anti-inflammatory cytokines in an opposite manner and this study suggests the involvement of H_3, and possibly H_4 receptors, in the pathogenesis of ONT.

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P26 COMPARATIVE mRNA EXPRESSION PROFILING IN NON-STIMULATED VERSUS H_4R-STIMULATED HUMAN LYMPHOCYTES

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The H_4R contributes to the regulation of major immune functions. In antigen presenting cells, the down-regulation of pro-inflammatory cytokines and chemokines has been observed. In contrast, on human lymphocytes, in particular on CD4+ T cells, it has been shown that the H_4R is up-regulated on Th2 polarized T cells and its activation leads to an increase in expression of the pro-inflammatory cytokine IL-31. In human Th17 cells, the stimulation with histamine or a H_4R agonist enhanced the production of IL-17.

In order to get a more comprehensive insight into cellular activities of human lymphocytes in response to H_4R stimulation, we performed microarray-based mRNA expression profiling, using the Whole Human Genome Oligo Microarray (G4845A, ID 026652, Agilent Technologies) which contained 44495 oligonucleotide probes covering roughly 27390 human transcripts. For this purpose, we treated human Th2 polarized T cells, CD8+T cells and natural killer cells (NK cells) with the selective H_4R agonist St1006 and compared the mRNA expression profiles with non stimulated control cells of the same origin.

We detected 13 genes which were differentially regulated in the Th2 cell group, 79 genes in the CD8+T cell group and 17 genes in the NK cell group. Interestingly, gene expression profiling shows no overlap of genes induced via H_4R in these three different lymphocyte subtypes. Regarding the Th2 cells, no influence of the H_4R stimulation on the expression of those cytokines, chemokines and transcription factors which characterise the classical Th2 phenotype has been observed. For the CD8+ T cells and the NK cells the microarray analysis revealed e.g. the up-regulation of particular chemokine receptors and chemokines.

Selected targets which were identified by this study will be investigated more thoroughly by qRT-PCR and protein analysis to further clarify the potential of targeting the H_4R for therapeutic benefit in a wide range of immune disorders.

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P27 SPECIFIC HISTAMINE H_4 RECEPTOR ANTAGONISTS ACT AS POTENT MODULATORS OF THE MAMMALIAN VESTIBULAR FUNCTION

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Histamine is a naturally occurring biological amine that exerts a range of effects on many physiological processes through the activation of four different G protein-coupled histamine receptors (H_1R-H_4Rs). In addition to their localization in various cells of the immune system, H_1R-H_4Rs are also found in the central and/or peripheral nervous system. For instance, recent work has shown that the H_3R is expressed in mammalian vestibular neurons. In addition, the H_3R antagonist, betahistine, routinely used in the treatment of vertigo can act peripherally by inhibiting the afferent discharge recorded from the vestibular nerve in lower vertebrates. We aimed to further explore the expression and role of H_1R-H_4R in afferent neurons from Scarpa's ganglion in the rat by combining single cell RT-PCR and immunohistochemistry experiments, as well as pharmacological and behavioral tests. Using this multidisciplinary approach we first confirmed the expression of H_1 and H_3 receptors and further demonstrated the localization of the H_4R in rat primary vestibular neuron somata. *In vitro*, whole cell patch-clamp recordings from P4-P7 cultured rat vestibular neurons revealed strong and reversible inhibitory effects on evoked action potential firing by H_4R antagonists JNJ10191584 and JNJ7777120 with IC_{50}s in the micromolar range. *In vivo*, the effect of these antagonists on experimentally induced severe vestibular deficits were evaluated in different animal models using a rating system based on multiple criteria: circling, head bobbing and head tilt, tail hanging and air righting reflexes. Each H_4R antagonist significantly alleviated the induced vestibular deficits by 20-30%. By contrast, neither of the H_3R antagonists tested as reference compounds had significant effects. The present study demonstrates the role of H_4R antagonists in modulating vestibular function and suggests that they are strong candidates for a novel, highly efficient treatment of vertigo crisis caused by peripheral vestibular dysfunction.

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P28 DIFFERENTIAL CHANGES IN H_4R EXPRESSION IN ACUTE AND CHRONIC INFLAMMATORY PAIN MODELS

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The histamine H_4 receptor (H_4R) is a G-protein coupled receptor that is primarily expressed on cells involves in inflammation and immune responses such as eosinophils, mast cells, dendritic cells and T-cells. It has been postulated to have immunomodulatory functions in these cells such as activation, migration and production of chemokines and cytokines[1]. Immunohistochemical studies from our laboratory and others have revealed the expression of the receptor on sensory neurons, small and medium sized diameter neurons and lamina I and II of the spinal cord suggesting a potential new target for the modulation of pain. The H_4R antagonist JNJ7777120, exhibits analgesic effects in Complete Freund's adjuvant (CFA) pain models[2]. The aim of the present study was to investigate the changes in H_4 receptor expression that occur in the acute (24 hours) and chronic (16 days) phases of Complete Freund's adjuvant induced inflammation as compared to non-treated animals (in collaboration with GSK (UK) and Pfizer (UK) (UK Home Office approved methods), respectively. Our

hypothesis was that H_4 receptor protein would be differentially altered during the acute and chronic phases of inflammatory-induced pain.
Using our well validated anti-hH_4 receptor antibody which cross-reacts with rat tissue[3], immunoblot analysis of skin and spinal cord tissue samples obtained from CFA-induced acute and chronic inflammatory rat pain models showed an apparent *upregulation* in the expression of H_4 receptors at the skin, but not the spinal cord. In contrast, this apparent up-regulation was not observed later in the chronic phase in the skin, but an apparent *down-regulation* of expression was seen in the spinal cord. This indicates both topological and temporal differences in H_4R expression occur in chronic inflammatory pain states.

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P29 EFFECTS OF 2,4-DIAMINOPYRIMIDINE H_4R LIGANDS ON THE HISTAMINE LEVELS IN THE NORMAL AND INFLAMED RAT CONJUNCTIVA

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The novel concept of the immunomodulatory role of histamine (HA) is supported by the primary distribution of the recently identified HA H_4 receptor (H_4R) in immune cells. This study aimed to investigate the effects of 2,4-diaminopyrimidine derivatives with different efficacies on the putative H_4R-mediated automodulatory HA function in the normal and inflamed rat conjunctiva. Male Wistar rats of 300-350g were maintained under controlled conditions. A 10μl drop of the 4-diaminopyrimidines ST-994, ST-1006 and ST-1012 (0.1-10mM dissolved in normal saline) was applied topically into the lower conjunctival fornix of one eye, either alone or after challenge with the mast cell degranulator C48/80 (100mg/ml). The contralateral eye served as the respective control, while ketotifen was used to validate the experimental model of C48/80-induced conjunctivitis. The animals were sacrificed 20 min after compound administration, the conjunctivae were removed (licence K/2889/11 EL) and HA levels were determined fluorometrically. Topical administration of the H_4R neutral antagonist ST-994 resulted in dose-related increases in the conjunctival HA content, while the partial agonist ST-1006 and the inverse agonist ST-1012 tended to decrease HA levels at high and low doses, respectively. Interestingly, ST-1012 failed to reverse the conjunctival response upon challenge with C48/80, similar to the previously reported effect of the H_4R antagonist JNJ7777120 in C48/80-induced conjunctivitis. These data point to a constitutive H_4R-meditated automodulatory HA function in the rat conjunctiva. The connection to immediate mast cell degranulation and the elucidation of the components intersecting H_4Rs and automodulatory HA functions under normal and inflammatory conditions is currently under intense investigation.

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P30 MICROSATELLITE REPEAT EXPANSIONS IN THE HUMAN HISTAMINE 4 RECEPTOR GENE

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Microsatellite repeat expansions have been implicated in a number of neurological disorders in humans, e.g., Fragile X syndrome, Huntington's disease and Kennedy's disease. Both loss-of-function and gain-of-function mechanisms are recognised from expanded trinucleotide repeats, and such microsatellites can be located in both coding and non-coding regions of the gene. This study targets genetic polymorphism in the promoter region of the human histamine 4 receptor (hH_4R) which contains a variable number of tandem repeat polymorphism (VNTR). This VNTR is a CAA triplet repeat expansion and ranges from 10 to 19 repeats (10R and 19R, respectively). In preliminary unpublished data it was indicated that the trinucleotide repeats 10R, 13R, 14R and 15R, are more common in schizophrenia patients. The aim of this study was to investigate the association of this genetic variation to schizophrenia and to assess promoter activity resulting from this polymorphism. Linkage disequilibrium (LD) analysis was carried out on HapMap samples between the VNTR in the promoter region and known Single Nucleotide Polymorphisms (SNPs) at this locus. The SNPs at hH_4R were tested for association with schizophrenia as part of a genome wide association studies (GWAS) conducted by the International Schizophrenia Consortium (ISC). Genotyping showed that the 13R allele is in high LD with rs17797945, while the 10 microsatellite is in high LD with rs628764 and rs615283. The SNP rs17797945 is weakly associated with schizophrenia in the ISC GWAS (P 0.007876). The promoter region including the variations in VNTR have been cloned and are currently being subcloned in a pGL3 promoter-less vector for assessment of promoter activity. This assay will indicate the functional impact this length polymorphism has on gene expression.

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P31 HISTAMINE MODULATES SALIVARY SECRETION AND DIMINISHES THE PROGRESSION OF PERIODONTAL DISEASE IN RAT EXPERIMENTAL PERIODONTITIS

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We have recently reported that experimental periodontitis reduced methacholine-induced submandibular gland (SMG) salivary secretion. The aim of the present study was to determine whether histamine could prevent SMG impairment produced by experimental periodontitis. Bilateral experimental periodontitis was induced for two weeks and histamine treatment (0.1 mg/kg, s.c.) was initiated 5 days before the end of the experimental period in male rats. The histamine effects on periodontitis-altered functional and histological parameters of SMG and on periodontal bone loss were evaluated.

Histamine treatme t
partially reversed the metacholine-induced salivation reduction produced by experimental periodontitis while preventing SMG histological damage. Histamine effect on SMG was associated with an increased proliferation rate (2.2 ± 0.3 vs. 0.2 ± 0.2, proliferative cells per field, P<0.001). Furthermore, histamine completely prevented the enhanced experimental periodontitis-induced apoptosis (1.0±0.4 vs. 60.9 ± 4.6 apoptotic cells per field, P<0.001). The protective effect exerted by histamine on SMG functionality is associated with the attenuation of the lingual and vestibular bone loss (0.66±0.04 vs. 0.97±0.06 mm; P<0.001) and the reduction of the increased prostaglandin E2 in gingivia of rats with experimental periodontitis.

Based on the evidence presented, we conclude that histamine is able to reduce periodontitis-induced damage on SMG and bone structure. Although further studies are needed to fully understand the role of histamine in periodontal disease, the present results suggest that this compound deserves to be studied as a potential agent to diminish periodontitis in a prospective study.

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P32 HISTAMINE IN PAROXYSMAL ATRIAL FIBRILLATION (AF): RESULTS FROM AN UNSELECTED POPULATION AT A TERTIARY UNIVERSITY EMERGENCY UNIT

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Since its discovery in 1910 several cardiac effects have been described for the biogenic amine histamine, including chronotrophic effects, vasodilation of coronary arteries, induction of arrhythmia etc. The present study investigated plasma histamine levels in patients with paroxysmal atrial fibrillation.

From 40 patients with paroxysmal atrial fibrillation, blood was drawn immediately after entry into the emergency unit before application of any therapy. Blood was stored on ice and centrifuged within 10 minutes for plasma histamine detection (IBL Hamburg). Plasma histamine was determined in duplicate and compared with 20 non-allergic controls and is expressed as ng histamine/ml x m^2 body surface area (BSA).

Plasma histamine in controls was 0.24 $\pm$ 0.11 (mean + SD) with a median of 0.26 (0.17 – 0.32; 25 – 75th percentile, Gaussian distribution). Thus, the normal range of plasma histamine levels was calculated as mean + 2SD with < 0.46.

Patients with AF did not significantly differ from controls, but showed a higher mean plasma histamine level of 0.52 $\pm$ 0.74 with a median of 0.31 (0.19 – 0.45). Interestingly, 8 of the 40 patients with AF (20.0%) exceeded the normal range of plasma histamine levels (> 0.46) when admitted to the emergency unit.

Among patients with paroxysmal AF a subpopulation of individuals could be detected with significantly elevated plasma histamine levels at admission. Thus, it might be considered that elevation of systemic or cardiac histamine levels may induce or provoke acute AF in these patients. Application of H_1- and H_2-antihistamines was beneficial in single patients to restore a regular sinus rhythm and further investigations have to focus on the etiology of plasma histamine elevations in this subpopulation (e.g. acute -, chronic elevations, allergic or inflammatory causes etc.)

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P33 THE EFFECTS OF HISTAMINE AND 4-METHYLHISTAMINE ON THE OXIDATIVE BURST OF HUMAN LEUKOCYTES

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Histamine plays an important role in immune system disorders and allergies. Since studies about the relation of the newly discovered histamine H_4 receptor with functional properties of phagocytes and phagocyte-derived reactive oxygen species generation are very rare, we focused on evaluating

histamine effects on functional activity of human leukocytes and on antioxidant properties of histamine.
Heparinized blood from healthy human volunteers was obtained by antecubital venipuncture. Total leukocytes including polymorphonuclear leukocytes were isolated and their ability to produce reactive oxygen species after histamine or its H_4 receptor agonist 4-methylhistamine treatment was tested by luminol-enhanced chemiluminescence analysis. Antioxidant properties of histamine and 4-methylhistamine were measured by luminol-enhanced chemiluminescence and fluorescence analyses.
Histamine and 4-methylhistamine increased the spontaneous oxidative burst in whole blood phagocytes. Similarly, both compounds increased the oxidative burst of whole blood phagocytes activated by opsonized zymosan particles or phorbol myristate acetate. On the other hand, neither of the compounds affected the oxidative burst in activated isolated leukocytes. The spontaneous oxidative burst of isolated leukocytes was even decreased by both compounds. Our results confirmed that neither histamine nor 4-methylhistamine have any antioxidant potential against reactive oxygen species.
It can be concluded that histamine interacted with immune system cells, especially with phagocytes. The increase in local concentrations of histamine could augment phagocyte-derived oxidative stress. H_4 receptors could at least partially play a role in this phenomenon.
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P34 MAST CELLS, ENDOTHELIAL PROLIFERATION AND CAPILLARY TUBE FORMATION IN MYOCARDIA OF PATIENTS WITH END-STAGE PRIMARY DILATED OR ISCHEMIC CARDIOMYOPATHY

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Inflammation is an important component in the pathogenesis of many common cardiovascular diseases, including ischemic (ICM) and primary dilated (PDCM) cardiomyopathy. The mast cell has an established position as a source of inflammatory and pro-proliferative mediators. The study aim was to examine whether there are differences in the number and distribution of mast cells, and in their relation to capillary endothelial proliferation and capillary tube formation in myocardium and subendocardial layer of patients with end-stage PDCM vs. ICM.
Myocardial specimens, without degeneration or tissue necrosis, from the anterior wall of 26 heart recipient patients (14 ICM, 12 PDCM) were obtained. H&E staining and immunohistochemistry, using anti-mast cell tryptase and anti-CD31, were employed. Quantitative analysis of the protein expression was performed by the MultiScan Base v.8.08 Image Analysis System. Tryptase-positive mast cells number and area, CD31-positive capillary endothelium area and capillary lumen were estimated in the myocardium and subendocardial layer in 65.739 μm^2 microscopic field. Values are presented as mean ± SD. For statistical evaluation, U Wilcoxon-Mann-Whitney test and Spearman range correlation coefficient were applied.
In myocardia of patients with end-stage PDCM, in comparison with ICM patients, an increased numerical density (0.51 ± 0.14 vs. 0.32 ± 0.16) and area (44.33 ± 15.41 vs. 29.71 ± 13.98 μm^2) of mast cells and excessive capillary endothelial cells proliferation with defect of capillary tube formation, respectively, was found. Microvessel lumen and endothelium area quotients were in PDCM always below 1 (0.53 ± 0.26), while above 1 (2.79 ± 1.53) in ICM patients. CD31-positive endothelium area correlated with tryptase-positive mast cell area in both groups. No differences were found in mast cell density in the subendocardial layer.
This preliminary study suggests that the mast cells in myocardium may be responsible for excessive capillary endothelial proliferation in tandem with defects in capillary tube formation, as expressed

by undeveloped lumen in primary dilated vs. ischemic cardiomyopathy patients. These observations may have therapeutic significance.

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P35 *Src* PROTEIN INVOLVEMENT IN HISTAMINE - INDUCED MDA-MB-231 CELLS MIGRATION

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We have previously demonstrated that cell migration and invasion of human breast carcinoma MDA-MB-231 cells were stimulated by low doses (0.5-1 µM) of histamine (HA), while inhibited by concentrations greater than 10 µM. These opposite actions are mediated by different intracellular hydrogen peroxide (H_20_2) levels. *c-Src* protein is activated by phosphorylation and this is critical for cell migration and cancer progression to metastasis. This protein phosphorylates substrates such as beta-catenin which translocates to the nucleus and modulates the expression of genes related to the epithelial mesenchymal transition (EMT). The aim of this work was to study the role of the *c-Src* protein in HA-induced migratory response of MDA-MB-231 cells.
We used a *c-Src* specific inhibitor, PP2, in a concentration which had no effect on cell proliferation. 1 µM PP2 blocked the stimulatory action of low doses of HA in cell migration and invasion as determined by transwells units coated or not with matrigel®. PP2 also blocked increase in further EMT markers (gelatinolytic activity and alpha smooth muscle actin expression) induced by 1 µM HA. An increase in *c-Src* phosphorylation was observed by western blot with 1 µM HA or exogenous 0.5 µM H_20_2 while a decrease was registered when HA was >10 µM or 5 µM H_20_2 were used. Catalase treatment reversed those effects.
The expression of beta-catenin protein was evaluated by immunostaining and Western blot. There was a higher nuclear and perinuclear expression with 1 uM HA. Conversely, the combined treatment of 1 µM HA plus 1 µM PP2 produced a cytoplasmic and nuclear beta catenin expression similar to non-treated cells. Low doses of HA also inactivated GSK-3beta, an enzyme involved in cytoplasmic degradation of beta catenin, favoring its nuclear translocation.
In summary, our results indicate that HA modulates the migratory and invasive capacity of MDA-MB 231 cells through a singular GPCR signaling pathway involving a H_2O_2-induced *c-Src* phosphorylation.

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P36 MODULATION BY HISTAMINE OF β_2 ADRENOCEPTOR-MEDIATED cAMP ACCUMULATION IN COS-7 CELLS: A POSSIBLE ALLOSTERIC ACTION

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Our previous data suggested an allosteric action of histamine to modulate cAMP formation induced by the activation of β_2 adrenoceptors endogenously expressed by human prostate cancer cells DU-145 (Ramos-Jiménez et al., 2007). In this work we have studied further this issue by combining functional assays with heterologously-expressed human β_2 adrenoceptors (hβ_2-ARs) and molecular modeling.
In COS-7 cells transiently transfected with the hβ_2-AR (10.4 $\pm$ 1.1 pmol/mg protein), incubation with histamine had no effect on basal cAMP accumulation but enhanced the response to the β-AR agonist isoproterenol (153 $\pm$ 15% of control response) in a concentration-dependent manner (EC_{50} 6.2 µM, pEC_{50} 5.21 $\pm$ 0.24). In contrast, the inhibition by isoproterenol of [^{3}H]-dihydroalprenolol binding was

not modified by histamine (p*K*i 5.99 ± 0.20 and 5.95 ± 0.10 in the presence and the absence of 10 μM histamine, respectively). Modeling on the hβ_2-AR showed the presence of three sites capable to bind imidazol-contaning drugs, and two of these sites had theoretical affinities (2-3 μM) in the range of histamine modulation of cAMP accumulation observed in transfected cells.
Taken together, these data provide further support for an allosteric action of histamine to modulate the intracellular signaling of human β_2 adrenoceptors.

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P37 RADIOPROTECTIVE POTENTIAL OF HISTAMINE ON RAT SMALL INTESTINE AND UTERUS

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Based on our previous data which showed the histamine radioprotective effect on mouse small intestine, bone marrow and salivary glands, in the present work we aimed to improve our knowledge about the histamine radioprotective potential by investigating its effect on reducing ionising radiation-induced injury in the rat small intestine and uterus. Rats were divided into 4 groups. Histamine and histamine-5Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg) starting 24 hours before irradiation. Histamine-5Gy and untreated-5Gy groups were irradiated with a dose of whole-body Cesium-137 irradiation. Three days post irradiation animals were sacrificed and tissues were removed, fixed, stained with haematoxylin and eosin and histological characteristics were evaluated. Proliferation and apoptosis markers were studied by immunohistochemistry. Results demonstrate that histamine treatment reduced radiation-induced mucosal atrophy, oedema and vascular damage produced by ionising radiation, increasing the number of crypts per circumference (239 ± 12 vs. 160 ± 10, P<0.01). This effect was associated with a reduction of radiation-induced intestinal crypts apoptosis. Furthermore, radiation-induced flattening of the endometrial surface, depletion of deep glands and reduced mitosis, effects that were completely blocked by histamine treatment. The expression of a proliferation marker in uterine luminal and glandular cells was markedly stimulated in histamine treated and irradiated rats. The evidence indicates that histamine is a potential candidate as a safe radioprotective agent that may increase the therapeutic index of radiotherapy for intra-abdominal and pelvic cancers. However, its efficacy needs to be carefully investigated in prospective clinical trials.

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P38 COMPARISON OF THE PHARMACOLOGICAL AND SIGNALING PROPERTIES OF WILD-TYPE AND A280V MUTANT HUMAN HISTAMINE H_3 RECEPTORS EXPRESSED IN CHOK1 CELLS

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Histamine H_3 receptors (H_3Rs) modulate at the pre-synaptic level the release of histamine and other neurotransmitters. In a patient suffering from the Shy-Drager syndrome an Ala to Val exchange at amino acidic position 280 (A280V) in the third intracellular loop was identified.[1] Further, this receptor polymorphism has recently been described as a risk factor for migraine in a Mexican population.[2] The third intracellular loop of G protein-coupled receptors is crucial for G protein-coupling and this variation may thus be relevant for the signaling triggered upon receptor activation. In this work, we set out to compare the pharmacological and signaling properties of wild-type and A280V mutant human H_3Rs expressed in CHOK1 cells.

The A280V mutant receptor was generated by overlapping extension PCR amplification using as template the human H_3R of 445 amino acids (hH_3R_{445}) sub-cloned into the vector pCIneo, and verified by automated sequencing. The wild-type and mutant hH_3Rs were stably expressed at similar densities (203 ± 34 and 173 ± 26 fmol/mg protein, respectively, n=5) by CHOK1 cells after lipofectamine-mediated transfection and geneticin-driven clone selection. There were no significant differences between both receptors in their affinities for H_3R ligands ([^{3}H]-N-α-methyl-histamine, histamine, R-α-methylhistamine and clobenpropit). The H_3R agonist R-α-methylhistamine inhibited forskolin-induced cAMP accumulation but the maximum response attained in cells expressing the wild-type hH_3R was significantly larger (students t-test) than that corresponding to the mutant receptor (-43.3 ± 6.6% and -24.5 ± 2.7%, respectively, n=8), while the pIC_{50} values were not different (-8.77 ± 0.17 versus 8.42 ± 0.14).

These preliminary data indicate that the A280V mutation modifies the signaling properties of the human H_3 receptor without altering agonist binding.

[1] P. Wiedemann et al., *Neural Transm*. 109: 443-453, 2002.
[2] R.O. Millán-Guerrero et al., *Arch. Med. Res.* 42: 44-47, 2011.

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P39 THE TH-1-ASSOCIATED CYTOKINE CXCL10/IP-10 IS DOWNREGULATED IN MONOCYTES AND MYELOID DENDRITIC CELLS – AN EFFECT POSSIBLY MEDIATED VIA DIFFERENT HISTAMINE RECEPTORS IN BOTH CELL TYPES

F. Jantzen, T. Werfel, R. Gutzmer

Histamine has been shown to influence inflammatory processes by modulating the cytokine expression of antigen presenting cells (APC), e.g. by downregulation of the Th1-associated cytokine IL-12. In this study, we analysed the effects of histamine on CXCL10/IP-10 (interferon gamma inducible protein), - another Th1-associated cytokine produced by APC.

Monocytes and myeloid dendritic cells (mDC) were isolated from human PBMC. After pre-incubation with different histamine concentrations (10^{-5}–10^{-10} mol/l) or 4-methylhistamine (10^{-5} mol/l) the cells were stimulated with PolyI:C for different time periods (2 to 24 hours). Supernatants were analysed by protein array for the expression of several cytokines. The concentration of IP-10 in the supernatants was quantified by ELISA. To analyse which is the responsible histamine receptor we

pretreated the cells with different receptor antagonists for 30 minutes (H_2R: ranitidine, H_4R: JNJ7777120).
A pre-stimulation with histamine caused a significant decrease of PolyI:C-induced expression of CXCL10 in a time- and dose-dependent manner in monocytes and myeloid dendritic cells. We observed this effect in protein arrays and ELISA with cell supernatants. Additional blocking with specific H_2R and H_4R antagonists showed that the observed effect is solely mediated via H_2R in monocytes, but appears to be mediated also via H_4R in mDC.
Taken together our results show that the Th1-associated chemokine CXCL10 is downregulated by histamine in monocytes and mDC. There appear to be differences in the histamine receptor responsible for this effect between monocytes and mDC, which is currently under investigation. This represents a new mechanism how histamine fosters a Th2 milieu by downregulating Th1 cytokines.

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P40 HISTAMINE INACTIVATING ENZYMES IN BREAST CANCER CELL LINES

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There is increasing evidence to demonstrate that histamine plays a significant role in breast cancer progression since functional histamine receptors and L-histidine decarboxylase activity are present in breast tissue. In addition, histamine is increased in plasma and cancerous tissue derived from breast cancer patients compared to healthy group. As the importance of histamine catabolism has not been fully studied in breast cancer cells, the aim of the present work was to investigate the expression and activity of the histamine inactivating enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HMT) in two breast cancer cell lines (MDA-MB-231 and MCF-7) with different malignant characteristics. Furthermore, we evaluated whether histamine treatment could modulate cell expression and activity. The expression of these two enzymes was determined by RT-PCR and western blot while the activity was measured by a radiometric assay. Results show that DAO was not detected in either cell lines both at the mRNA and protein level. On the other hand, HMT enzyme was expressed in MDA-MB-231 and MCF-7 cells. In agreement with these results, DAO enzyme activity was not observed while HMT activity was detected in both cell lines. HMT activity seemed to be down-regulated in histamine treated cells (1.5 mU/mg vs. 6.7 mU/mg in untreated MDA-MB-231 cells; 1.7 mU/mg vs. 5.6 mU/mg in untreated MCF-7 cells). We conclude that HMT is the only histamine inactivating enzyme detected, suggesting a crucial role of this enzyme in histamine catabolism in breast cancer.

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P41 HISTAMINE ACTIONS IN THE NORMAL HUMAN FIBROBLAST CELL LINE CCD-1059Sk

J. Porretti, N. Mohamad, E. Badenas, M. Esnaola, E. Rivera, G. Martín, G. Cricco

We have previously demonstrated a high expression of histidine decarboxylase (HDC), the enzyme involved in histamine (HA) synthesis and therefore a high HA content in the human pancreatic

carcinoma cell line BxPC3. *In vivo*, HA produced a significant desmoplasia in nude mice bearing BxPC3 xenografts and also neoplastic cells colonizing lung parenchyma were observed. *In vitro*, conditioned medium of BxPC3 cells treated with (HA 1-10 µM) increased the number of CCD-1059Sk cells in S phase of cell cycle. The aim of this work was to evaluate HA action on cell proliferation, gelatinases expression and activity and cell motility in the normal human fibroblast cell line CCD-1059Sk.

A direct action of HA (1-10 µM) on fibroblast proliferation was observed employing bromodeoxyuridine (BrdU) incorporation technique. After 8h of HA treatment there was a significant rise in fibroblast cells number in S phase ($p<0.05$ vs non-treated cells). By RT-PCR, we determined the mRNA expression of H_1, H_2, H_3 and H_4 histamine receptors, the matrix metalloproteinase 2 (MMP2) and the tissue inhibitor of MMP (TIMP1) in this cell line. Immunostaining studies showed that CCD-1059Sk fibroblasts express cytoplasmic MMP2 protein, although no difference in MMP2 expression was observed between HA treated and non-treated cells. Gelatinolytic activity evaluated by zymography was significantly increased by 10 µM HA and reproduced by the H_1 histamine receptor agonist. 10 µM HA enhanced cell migration ($p<0.05$ vs non treated cells) assessed using transwell units.

In summary, we may conclude that exogenous HA exerts a direct action on the normal human fibroblasts CCD-1059Sk proliferation, gelatinolytic activity and motility, opening the perspective for future studies on the role of histamine in fibroblasts activation during the epithelial mesenchymal transition related to cancer progression.

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Publication of the EHRS meeting's proceedings

The proceedings of the meeting will be published in the journal Inflammation Research.
All papers will be published as abstracts and should confirm to the style of the journal printed pages.
All manuscripts must be emailed to the Publications Secretary (Gill Sturman)
by the **1st June** at the latest.
Manuscripts are to be submitted by email to *gill.sturman@virgin.net*.

Abstract Instructions

Please arrange the abstract as follows:

- **Title in boldface, in CAPITALS (Times New Roman 12).**
- *New line with (Times New Roman 12, in italics) giving the Author(s) name(s), separated by commas (initials separated by full stop only, space family name comma, repeating as necessary). The presenting author should be underlined. Do not add titles (e.g. Prof., Dr. etc.)*
- Leave the next line blank.
- Type the text in Times New Roman 11 on a new line without indentation.
- The abstract should be informative and of an appropriate scientific standard. It should include sections on Background; Methods; Results; Conclusions **(without the titles of the sections).**
- Tables and figures are **not** permitted.
- References are also **not** permitted.
- The length of the text should **NOT EXCEED 2000 characters** (with spaces).
- *Finally on a new line and in italics give with the name of the **institution of the corresponding author only** with the city, postal code and country.*
- *Then the email address of the corresponding author – again in italics.*

The International Anthem of the EHRS

The anthem is sung to the tune of Polly Waddle Doodle and new lines/verses are added each meeting.

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 Hanover 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.
Let us sing this song together / Histamine will last forever,
Singing Histaminosis all the day.

CHORUS

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

17. Then to Marburg we returned / ninety one and also learned
That histamine in surgery´s not good.
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 Seville, 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 Millennium 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.
András called two thousand two / and to Eger did we go
To a meeting in Hungary again.

23. In the year two thousand three / we did lots of tulips see
Now Henk Timmerman was host in Amsterdam.
And once again have we seen / all the fans of histamine
Singing Histaminosis all the day.

24. To lovely Bled we return / and once again we did learn
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. Back 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.
And to Durham we went then / in the year two thousand ten.
There with Paul near Cathedral did we stay.

27. Two thousand and eleven / and in Sochi it was heaven
When our Roman he did the Russian way
Then to **Belfast** the next year / it was lovely, Maddy dear
Irish meeting was excellent in May.

CHORUS

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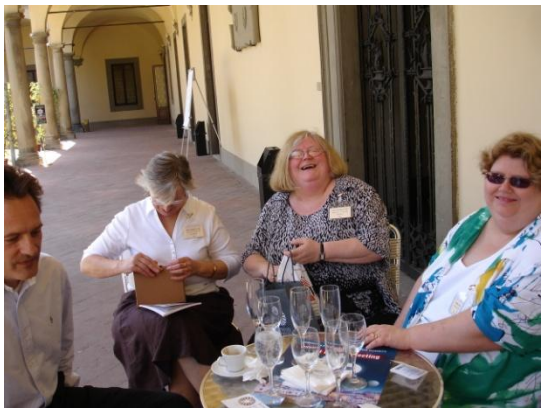
This meeting is dedicated to the memory of my younger sister Pauline Haines nee Ennis, who died suddenly and unexpectedly on December 3rd 2011. She had planned to organize and run the social programme for us.



Florence

Fulda