European Histamine Research Society
43rd Annual Meeting
May 7 - 10, 2014
Lyon France

PROGRAM
ABSTRACT BOOK

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<td>8.30-8.45</td>
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<td>GB West lecture Kazuhiro Yama Introduction by Pertti Panula</td>
<td>Arrival &amp; registration (14.00-20.00) EHRSCouncil meeting (16.00-18.00) Welcome Reception (19.00-22.00)</td>
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<td>8.45-9.15</td>
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<td>Plenary lecture Jean-charles Schwartz Introduction by Helmut Haas</td>
<td>Meeting of poster committees and jury (16.30-17.00) EHRSGeneral Assembly</td>
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Dear Histaminologists, dear friends,

Welcome to the 43rd Annual Meeting of the European Histamine Research Society which will be held in Lyon from the 7th to 10th May 2014. Some of you have seen Lyon for the occasion of the 1999 meeting, but the host institution is now University of Claude Bernard and the venue Domain St-Joseph is located 6 km from Lyon on the shoulder of the characteristic Fourvière Hill that dominates the very old and historical down town.

As usual, the conference will cover a wide range of histamine research fields from the brain to periphery. The past year has been an important year for the histamine research as the link between histamine and brain diseases has never been so clearly demonstrated, e.g., the number of histamine neurons seems to largely increase in narcolepsy whereas genetic deficiency of histidine decarboxylase likely causes Tourette syndrome.

We appreciate the high scientific standard of the submitted abstracts promising a very attractive scientific program. I thank particularly the invited speakers who will deal with the current major topics of histamine research. We will see firstly that the H3-receptor constitutes the brain therapeutic target for sleep disorders and that the H3-receptor inverse agonists, finally, are being introduced in sleep medicine. We will learn that hypothalamic sleep active neurons exert their REM sleep-promoting function via, in part, a GABAa mediated post-synaptic inhibition of the wake-promoting histaminergic neurons. We will find out that modern brain imaging allows demonstration of the functional role of histamine and molecular mechanisms involved under physio-pathological conditions in the human brain and will constitute a powerful tool for histaminergic drug development in this millennium. Moreover, we will appreciate that the long-sought interaction between histamine and dopamine is today substantiated by molecular, cellular, behavioral and soon therapeutic evidence. Finally, we will discover that, during immune reactions, salivary epithelial “non-professional” histamine-producing cells actually behave like “professionals” and maintain the homeostatic fine-tuning of the high-affinity histamine receptors!

Furthermore, eight sessions of oral presentations and four poster sessions will cover all fields of histamine research. We expect a very exciting meeting thanks to the active participation of all histaminologists attending the meeting. Besides the Science, I hope you will enjoy Lyon and our outing.
Capital of the three Gauls under the Roman Empire, Lyon is one of the oldest cities in France and Europe. The city is known for its historical and architectural landmarks and is a UNESCO World Heritage Site. Lyon was historically known as an important area for the production and weaving of silk. It has also a significant role in the history of cinema due to Auguste and Louis Lumière, who invented the cinematography in Lyon. The city is also known for its famous light festival 'Fête des Lumières', earning Lyon the title of Capital of Lights. Legend says that the Virgin Mary saved the city from the plague and a statue was built to thank her. On the day it was erected, the whole city was lit by candles that its citizens had put at their windows. In modern times Lyon has developed a reputation as the capital of gastronomy in France, offering the choice between its famous “Bouchons” and over forty rated gastronomical restaurants like the unique 3 stars chef Paul Bocuse.

Located in the central part of France where the River Rhône meets another long and quiet river the Saône, Lyon is also known by her third river, the Beaujolais wine which flows to all parts of the world every year. Today, Lyon is a large European metropolis of about 1.2 million inhabitants and has become a major centre for culture, universities and research as well as for chemical, pharmaceutical, and biotech industries.

The day of our outing, we will firstly ascend to the Fourvière Basilica and enjoy the free time visiting the Basilica and admiring the panoramic view of Lyon City. The visit will be followed by a guided tour of the Old Lyon and its “Traboules”. Then, we will be guided to the Maison des Canuts, a unique museum of Lyon silk industry. At the late afternoon, we will be out of Lyon for vineyard and cellar visit and wine tasting at Clos St Marc. Here in the cellar we will spend our evening around traditional gastronomic tables and enjoy the regional products under musical ambiance.

The scenery of the Lyon region is stunning with so many cultural and historical places to visit. If you can spend some extra time here, you will love it.

I look forward to meeting you in my adopted home.

Jian-Sheng Lin
## Previous EHRS Annual Meetings

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Committees

Scientific & Organizing Committee

Jian-Sheng LIN Chair (Lyon, Fr)

Colette BUDA (Lyon, Fr)

Françoise PERROT (Lyon, Fr)

Jean-Charles SCHWARTZ (París, Fr)

Michel DY (París, Fr)

International Advisory Committee

Paul CHAZOT, EHRS President (Durham, UK)

Anita SYDBOM, EHRS Past President (Stockholm, SE)

Pertti PANULA, EHRS Treasurer (Helsinki, FI)

Gill STURMAN, EHRS Publication secretary (Essex, UK)

Nick CARRUTHERS, EHRS Council Member (San Diego, USA)

Pierre CHATELAIN, EHRS Council Member (Bruxelles, BE)

Beatrice PASSANI, EHRS Council Member (Firenze, IT)

Astrid SASSE, EHRS Council Member (Dublin, IE)

Madeleine ENNIS (Belfast, UK)

Helmut HAAS (Dusseldorf, D)
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The EHRS Young Investigator Award Committee

Anita Sydbom (Stockholm, SE)
Bernard Gibbs (Kent, UK)
Helmut L Haas (Dusseldorf, D)

Poster Prize Committee

Madeleine Ennis (Belfast, UK) Chairman
Patrizio Blandina (Firenze, I)
Ilona Obara (Durham, UK)
Pertti Panula (Helsinki, Fi)
Jerzi Jochem (Bytom, PL)
Arianna Rosa (Torino, I)
Rob Thurmond (San Diego, USA)
Vanina Medina (Buenos Aires, AR)

Abstract Evaluation and Bursary Committees

Paul Chazot EHRS President
Anita Sydbom EHRS Past President
Pertti Panula EHRS Treasurer
Gill Sturman EHRS Publication secretary
Nick Carruthers EHRS Council Member
Jian-Sheng Lin EHRS Council Member
Pierre Chatelain EHRS Council Member
Beatrice Passani EHRS Council Member
Astrid Sasse EHRS Council Member
General Information

Location
Domaine Saint Joseph
38 allée Jean-Paul II
69110 SAINTE FOY LES LYON

Registration / Welcome desk
It is located in the Entrance Hall of the Domain. You will collect your delegate badge and conference documentation at the registration desk and then check in. Please note that meeting, restaurant, visit, exhibition areas are accessible only with your name badge.

Internet
Wireless internet access is available in the Domain. Login and password are provided in your delegate bag.

Working Language
The working language of the conference is English. No interpretation is provided in any other language.

Insurance
The organizers do not accept responsibility for individual medical, revel or personal insurance. Participants are strongly advised to take out their own insurance policies.

Certificate of attendance
All registered participants will be delivered a certificate of attendance.

Poster Area
The poster session will take place in the ground floor.

Lunch
Midday Lunch will be taken in a private area in the Domain restaurant.

Telephone
The international code for France is 33. Public phones accept credit cards and phone cards available in tobacconists, newsagents, post offices.

Emergency phone numbers
Medical care: the free number for hospital emergency (SAMU) is 15.
Police: The free number for the police is 17

Tourism Office
Pavillon du Tourisme - Place Bellecour - B.P. 2254,69214 LYON Cedex 2
Tel : 04.72.77.69.69 / http://www.lyon-france.com
The **Domaine Lyon Saint Joseph's** origins are closely linked to Lyon’s Catholic church.

The building was intended to increase the capacity of Lyon’s Diocesan Seminary, and was constructed between 1926 and 1928 on land acquired by the Diocese in 1860. Entirely constructed in concrete, including the roof structure, the architecture presents an innovative style, but also reflects the monastic tradition with its cloister.

The Saint Joseph Seminary came to the assistance of the neighbouring Saint Irénée Seminary in 1930, which was unable to accommodate all the seminarians. For forty years, the first two years of training in philosophy were conducted within its walls.

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**Local Public Transport**

Public transportation is running **from 5 am to midnight**.

For more information: [http://www.tcl.fr/](http://www.tcl.fr/)

To go downtown: Bus line C20

**Taxis**

- www.taxilyon.com: +33 4 72 10 86 86
- www.taxilyonnais.com: +33 4 78 26 81 81
- www.allotaxi.fr: + 33 4 78 28 23 23
MAY, 9th 2014 : LYON TOUR

**12.45**: Departure (bus) from the Conference site to the “Basilique de Fourvière”

**13.15**: Free time on site

![Basilique de Fourvière](image)

**14.00—16.00**: Guided Tour of the Old Lyon

**16.00—16.30**: Free Time in the Old Lyon

**16.30**: Departure (bus) from the Old Lyon to the “Maison des Canuts”, Museum of the Lyon Silk Industry

**17.00—18.00**: Guided tour in the Museum

![Maison des Canuts](image)

**18.00**: Departure (bus) from the Museum to “Clos St Marc”

**18.45**: Visit of the Vineyard and Cellar

Traditional Dinner with regional products in the Clos St Marc cellar.

**23.15**: Departure from the “Clos St Marc” to the Conference Site.
Invited speakers

Prof Jean-Charles SCHWARTZ, Bioprojet, Paris

« Clinical trials with Pitolisant, a wake-promoting H3-receptor inverse agonist »

Prof Olga A. SERGEEVA, Heine-Univ. Dusseldorf

« Histamine—dopamine interactions in health and disease »

Prof Kazuhiko YANAI, Tohoku Univ., Sendai

« Neuroimaging studies in humans on the histaminergic neuron system: past, present and future »

Prof Yrjo KONTTINEN, Biomedicum Helsinki

« Non-professional histamine producing cells and H4R in immune reactions in general and in the pathogenesis of Sjögren’s syndrome in particular »

Dr Antoine ADAMANTIDIS, McGill University Montréal

« The MCH ➔ Histamine connection: a possible modulatory circuit for REM sleep »
EHRS 2014- WEDNESDAY, 7TH OF MAY 2014

14.00 - 20.00 Arrival and registration
16.00 –18.00 EHRS Council meeting
19.00 - 22.00 Welcome Reception

EHRS 2014 - THURSDAY, 8TH OF MAY 2014

8.30 –9.00 Opening Session
   Jian-Sheng Lin (Chairman of the meeting)
   Paul L Chazot (President EHRS)
   Germain Gillet (UCBL Scientific Council Vice-President)
   Student Bursaries distribution

9.00 – 9.15 Honorary Member Ceremony

9.15 -10.00 Plenary lecture
L1 Jean-Charles Schwartz, Paris, France
   CLINICAL TRIALS WITH Pitolisant, A WAKE-PROMOTING H3-RECEPTOR INVERSE AGONIST
   Introduction by Helmut Haas

10.00-10.30 Coffee break and Group Photo

10.30-11.45 Oral session I: BRAIN HISTAMINE: FUNCTION AND BEHAVIOUR
   Chaired by Beatrice Passani and Bassem Sadek

10.30 - 10.45 O1
ROLE OF THE HISTAMINERGIC SYSTEM IN SHORT-TERM AND LONG-TERM MEMORY OF INHIBITORY ACTIVE AVOIDANCE (IAA)

10.45 - 11.00 O2
K. Ouk, C. Zhao, C. Buda, C. Anaclet, Y. Zhao, H. Ohtsu, M. Yanagisawa, J.S. Lin
THE ROLE OF THE HISTAMINERGIC AND OREXINERGIC SYSTEMS IN ANTICIPATORY WAKEFULNESS

11.00 - 11.15 O3
L. Seugnet, C. Anaclet, M. Perier, H. Akaoka, J.S. Lin
A TRANSCRIPTOMIC AND GENETIC APPROACH TO IDENTIFY MOLECULAR MECHANISMS UNDERLYING WAKEFULNESS

11.15 - 11.30 O4
Bassem Sadek, Amine Bahi, Stephan J. Schwed, Miriam Walter, Holger Stark
ANXIOLYTIC- AND ANTIDEPRESSANT-LIKE ACTIVITIES OF THE NOVEL AND POTENT TRIAZOLE HISTAMINE H3 RECEPTOR ANTAGONIST ST-1283

11.30 -11.45 O5
P.L. Chazot, R.M. Abuhamdah, A Ennaceur
HISTAMINE H4 RECEPTORS ARE NOT INVOLVED IN ANXIETY BEHAVIOURAL RESPONSES
Scientific Program

11.45-12.45 Oral session II: HISTAMINE NEURON: REGULATION AND INTERACTION

Chaired by Jian-Sheng Lin and Rushdie Abuhamdah

11.45-12.00
O6

L. Haas, R. De Luca, O.A. Sergeeva
GLUTAMATERGIC EXITATION OF HISTAMINERGIC NEURONS.

12.00-12.15
O7

P. Panula, S. Semenova, M. Sundvik, S. Rozov, Y-C Chen
DOPAMINERGIC REGULATION OF THE BRAIN HISTAMINERGIC AND HYPOCRETIN SYSTEMS IN ZEBRAFISH

12.15-12.30
O8

Y. Miura, T. Yoshikawa, T. Shibakusa, M. Sugita, K. Yanai
IMPORTANCE OF HISTIDINE INTAKE FOR HISTAMINERGIC NERVOUS SYSTEM

12.30-12.45
O9

THE INHIBITORY EFFECT OF HISTAMINE IN MOUSE PRIMARY MICROGLIA

12.45-13.45 Lunch

13.45-14.30 Plenary lecture
L2

Antoine Adamantidis, Montreal, Canada
THE MCH - HISTAMINE CONNECTION: A POSSIBLE MODULATORY CIRCUIT FOR REM SLEEP?
Introduction by Jian-Sheng Lin

14.30-15.15 Oral session III: HISTAMINE, HIPPOCAMPUS AND SENSORILE SYSTEM

Chaired by Paul Chazot and Susanne Mommert

14.30-14.45
O10

L. Yang, B. Zou, X. Xiong, C. Pascual, J.J. Xie, A. Malik, J.Z. Xie, T.Sakurai, X.S. Xie
HYPOCRETIN/OREXIN NEURONS CONTRIBUTE TO HIPPOCAMPUS-DEPENDENT SOCIAL MEMORY AND SYNAPTIC PLASTICITY IN MICE

14.45-15.00
O11

P.L. Chazot, M.A. Katebe, N. Gamper
THE HISTAMINE H4 RECEPTOR IS FUNCTIONALLY EXPRESSED ON RAT DORSAL ROOT GANGLIA NEURON AND GLIA SUBPOPULATIONS

15.00-15.15
O12

I. Obara, M.C. Medrano, A. Miles, L. Jiménez-Diaz, S. M. Géranton, S.P. Hunt
ATTENUATION OF ITCH SENSATION IN MICE BY INHIBITION OF THE MAMMALIAN TARGET OF RAPAMYCIN COMPLEX 1 (MTORC1) SIGNALING PATHWAY

15.15-15.45 Coffee break

15.45-17.15 Poster Sessions I and II
Poster Session I: HISTAMINE, FUNCTION AND DYSFUNCTION
Chaired by Patrizio Blandina and Ilona Obara

Poster Session II: BRAIN HISTAMINE, FUNCTION AND BEHAVIOR
Chaired by Pertti Panula and Jerzy Jochem
Scientific Program

17.15-18.45 Poster Sessions III and IV
Poster Session III: HISTAMINE: MOLECULAR SIGNALING
Chaired by Madeleine Ennis and Arianna Rosa
Poster Session IV: INFLAMMATION
Chaired by Rob Thurmond and Vanina Medina

18.45-19.15 Meeting of Poster Committees
19.30-21.30 Dinner

EHRS 2014 - FRIDAY, 9TH OF MAY 2014

8.30-9.15 GB West lecture
L3 Kazuhiko Yanai, Sendai, Japan
NEUROIMAGING STUDIES IN HUMANS ON THE HISTAMINERGIC
NEURON SYSTEM: PAST, PRESENT AND FUTURE.
Introduction by Pertti Panula

9.15 – 10.15 Oral session IV: INFLAMMATION AND ALLERGY
Chaired by Ralf Gutzmer and Przemyslaw Rzodkiewicz

9.15 – 9.30
O13 E. Schneider, R. Rignault, F. Machavoine, M. Dy
NEWS IN THE RELATIONSHIP BETWEEN HISTAMINE AND BASO
PHILS

9.30 – 9.45
O14 H. Ohtsu, A. Sato
SPECIFIC SENSITIZATION MECHANISM IN MOUSE NICKEL ALLERGY
AND THE ROLE OF HISTAMINE IN THIS MODEL

9.45 – 10.00
O15 B. Koether, F. Glatzer, S. Mommert, H. Stark, T. Werfel, R. Gutzmer
DIFFERENTIAL EFFECTS OF HISTAMINE H1R AND H2R VERSUS H4R
ON THYMIC STROMAL LYMPHOPOIETIN (TSLP) BY HUMAN
KERATINOCYTES

10.00 - 10.15
O16 H. Fukui, H. Mizuguchi, Y. Kitamura, N. Takeda
IMPROVEMENT OF SYMPTOMS WITH CORRELATIVE SUPPRESSION
OF ALLERGIC DISEASE-SENSITIVE GENE EXPRESSION

10.15-10.45 Coffee break and poster viewing

10.45-12.30 Oral session V: HISTAMINE: RECEPTOR AND LIGAND
Chaired by Rob Leurs and Holger Stark

10.45 – 11.00
O17 J. Felixberger, A. Strasser, M. Tanaka, S. Elz, G. Bernhardt, T. Ozawa, A.
Buschauer
BIASED AGONISM AT THE HISTAMINE H4 RECEPTOR:
CHIRALHISTAPRODIFEN DERIVATIVES SHOW FUNCTIONAL
SELECTIVITY FOR β-ARRESTIN RECRUITMENT

11.00-11.15
O18 M. S. Tichenor, J. M. Blevitt, H. Banie, R. L. Thurmond, D. K. La, J. D. Ven-
able, P. M. McGovern, G. M. Bacani, P. J. Dunford
H4 RECEPTOR AGONISTS ARE WELL TOLERATED IN MICE WITH NO
ADVERSE HEMATOLOGICAL FINDINGS
11.15-11.30  
**O19**


**EFFICACY AND SAFETY OF THE H4R ANTAGONIST JNJ-39758979 IN A PHASE 2A CLINICAL STUDY IN PATIENTS WITH ATOPIC DERMATITIS**

11.30-11.45  
**O20**

*D. Wifling, K. Löffel, U. Nordemann, G. Bernhardt, S. Dove, R. Seifert, A. Buschauer*

**IN SEARCH FOR KEY AMINO ACIDS DETERMINING THE HIGH CONSTITUTIVE ACTIVITY OF THE HUMAN HISTAMINE H4 RECEPTOR**

11.45-12.00  
**O21**

*H. Engelhardt, S. Nijmeijer, H.F. Vischer, C. de Graaf, I.J.P. de Esch, R. Leurs*

**TRIAZOLOQUINOXALINES AS POTENT HISTAMINE H4 RECEPTOR LIGANDS**

12.00-12.15  
**O22**

*N. I. Carruthers*

**PRECLINICAL CHARACTERIZATION OF THE HISTAMINE H3 RECEPTOR ANTAGONIST BAVISANT (JNJ-31001074) AND DETERMINATION OF DRUG LEVELS REQUIRED FOR ROBUST TARGET ENGAGEMENT IN HEALTHY VOLUNTEERS.**

12.15-12.30  
**O23**

*A. Rayan*

**DISCOVERY OF NATURAL BASED & CARDIOSAFE LIGANDS OF HUMAN H4R USING COMPUTERIZED TECHNIQUES**

12.30-19.00  
**Outing, lunch aboard**

19.00-22.00  
**Outing Dinner**

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**EHRS 2014 - SATURDAY, 10TH OF MAY 2014**

9.00-9.45  
**Plenary lecture**

*L4*

Olga A Sergeeva, Dusseldorf, Germany

**HISTAMINE - DOPAMINE INTERACTIONS IN HEALTH AND DISEASE**  
*Introduction by Patrizio Blandina*

9.45-10.45  
**Oral session VI: HISTAMINE, CELLULAR SIGNALING AND IN VIVO REGULATION**  
*Chaired by Astrid Sasse and Linda Kay*

9.45-10.00  
**O24**

*Y. Miura, T. Yoshikawa, F. Naganuma, T. Nakamura, T. Iida, R. Harada, A. Mohsen, K. Yanai*

**ANALYSIS OF MOUSE POLYSPECIFIC TRANSPORTERS**

10.00-10.15  
**O25**

*N. Kagermeier, U. Nordemann, G. Bernhardt, G. König, A. Buschauer*

**DIFFERENTIAL SIGNALING PATHWAYS OF THE hH1R AND THE hH2R**

10.15-10.30  
**O26**

*B.F. Gibbs, L. Calzolai, V.V. Sumbayev*

**SPECIFIC TARGETING OF HUMAN ALLERGIC EFFECOR CELL FUNCTION USING GOLD-BASED NANOMATERIALS**
10.30-10.45

**O27**


**IMPACT OF ORAL VITAMIN C ON HISTAMINE LEVELS AND SEASICKNESS**

10.45-11.15 Coffee break and poster viewing

11.15-12.15 Oral session VII: HISTAMINE H3 AND H4 RECEPTORS

*Chaired by Michel Dy and Pierre Chatelain*

11.15-11.30

**O28**


HISTAMINE H3 RECEPTOR LIGANDS RETAINS INTRAOCULAR PRESSURE (IOP)-LOWERING ACTIVITY AND ENHANCES VASCULAR FLOW IN RETINIC ARTERY IN GLAUCOMA MODELS.

11.30-11.45

**O29**

E. Veglia, C. Grange, A. Pini, G. Camussi, P.L. Chazot, A.C. Rosa

HISTAMINE H3 RECEPTOR EXPRESSION IN THE HUMAN KIDNEY

11.45-12.00

**O30**


FUNCTIONAL IDENTIFICATION OF THE H4R IN THE HUMAN KIDNEY

12.00-12.15

**O31**

D. Martinel Lamas, E. Cortina1, E. Carabajal, J.C. Perazzo, E.S. Rivera, V.A. Medina

HISTAMINE H4 RECEPTOR AGONISTS ARE SYNERGISTIC WITH DOXORUBICIN IN BREAST CANCER

12.15-13.15 Lunch

13.15-14.00 Plenary lecture

L5

Yrjö Konttinen, Helsinki, Finland

NON-PROFESSIONAL HISTAMINE PRODUCING CELLS AND H4R IN IMMUNE REACTIONS IN GENERAL AND IN THE PATHOGENESIS OF SJÖGREN’S SYNDROME IN PARTICULAR

*Introduction by Paul Chazot*

14.00-15.15 Oral session VIII: EHRS YOUNG INVESTIGATOR AWARD SYMPOSIUM

*Jury and Chair: Anita Sydbom, Bernard Gibbs, Helmut Haas*

14.00 – 14.15

**O32**

J. Vanhanen, S. Nuutinen, P. Panula

HISTAMINE H3-RECEPTOR DEPENDENT MEDIATION OF BRAIN DOPAMINERGIC REGULATION IN MICE

14.15- 14.30

**O33**

M.D. Sanna1, N. Galeotti1, L. Lucarini1, C. Lanzi1, H. Stark2, C. Ghelardini1, E. Masini1

HISTAMINE H4 RECEPTOR ACTIVATION INDUCES ANTINOCEPTION IN A NEUROPATHIC PAIN MODEL

14.30- 14.45

**O34**

S. Nijmeijer, H.F. Vischer, C. de Graaf, I.J.P. de Esch and R. Leurs

FUNCTIONAL FRAGMENT SCREENING AT GPCRS: TOWARDS SIGNALLING-BIASED FRAGMENTS.
Scientific Program

O35  H2R-MEDIATED MODULATION OF TLR2-INDUCED CYTOKINE RESPONSES ARE AMELIORATED IN PATIENTS WITH IBD

O36  THE ACTIVATION OF THE SEPTUM LATERAL INCREASED THE RELEASE OF BRAIN HISTAMINE

15.15-17.00  Coffee break and final poster viewing
16.30-17.00  Meeting of poster committees and jury
17.00-18.30  EHRS General Assembly
20.00  Farewell Dinner
Invited Lectures
CLINICAL TRIALS WITH PITOLISANT (WAKIX®) A WAKE-PROMOTING H3-RECEPTOR INVERSE AGONIST

Jean-Charles Schwartz
Bioprojet, Paris, St Grégoire, France. jc.schwartz@bioprojet.com

The best established function of histaminergic neurons is their role in the maintenance of wakefulness. The development of inverse agonists of the histamine H3 receptor (H3R), a class of compounds enhancing their activity and the release of histamine in the whole CNS, first provided a tool to confirm this role in animals (Lin et al 1990) and, as a consequence, it became apparent that these agents could be useful in human disorders of vigilance.

The first H3R inverse agonist to be introduced in therapeutics is pitolisant, a non-imidazole compound of nanomolar potency, displaying high oral bioavailability, high brain penetration and an excellent safety profile in animal toxicity including carcinogenicity studies; its wake-promoting activity was shown in cats, rats and mice, including in orexin -/- mice, a reliable model of narcolepsy (Ligneau et al 2007; Lin et al 2008).

Phase I trials in healthy volunteers confirmed the good safety profile, showed a T1/2 of ~10h, compatible with a once-a-day administration without affecting nocturnal sleep and there were clear indications of enhanced wakefulness and attention (Schwartz, 2011).

A series of Phase IIa trials were performed in a rather large variety of potential neuropsychiatric indications based upon suggestions gathered from more or less reliable preclinical models: epilepsy, ADHD, schizophrenia, dementias (Lewy bodies dementia), excessive daytime sleepiness (EDS) in narcolepsy, Parkinson’s disease and obstructive sleep apnoea (OSA). Among these proof-of-concept trials, the EDS pathologies provided the most promising positive signals and these pathologies were therefore selected for further developments.

Dose-finding studies in EDS showed that 20 mg tablets taken once-a-day corresponded to the Minimal Effective Dose but 40 mg o.d. was equally well tolerated.

Three pivotal trials in narcolepsy, a disease for which pitolisant has received an orphan drug designation from both the EMEA and FDA, were performed using individual flexible dose adjustment, and found successful (HARMONY I, Ibis and III). Significant EDS improvement was evidenced using either the Epworth Sleepiness Scale, which assesses EDS in a variety of current life circumstances, or the MWT (Maintenance of Wakefulness Test), a laboratory test measuring the time a patient remains awake in a dark and quiet environment. Significant improvement was also recorded in the SART (Sustained Attention to Response Task) measuring the level of attention. Interestingly, pitolisant decreased by over 60% the frequency of cataplexy attacks (Dauvilliers et al, 2013), an effect not seen with psychostimulants e.g. amphetamines or modafinil, but which is consistent with data on orexin knockout mice (Lin et al 2008). The efficacy of pitolisant on EDS did not differ significantly from that of modafinil but tolerance was better, namely regarding withdrawal symptoms which were totally absent after the of pitolisant treatment, in agreement with data in a variety of animal models indicating lack of drug abuse potential (Uguen et al 2013); only minor adverse events were recorded among the patients having received pitolisant (over 1,000) some for up to 1 year: headache, nausea, abdominal discomfort...

In OSA, Phase IIa and IIb suggested also clear improvement of EDS and two pivotal trials are currently being completed.

Hence Wakix® appears as a novel, safe and efficient wake promoter without the drawbacks of psychostimulants. In addition, Wakix® displays clear anticataplectic properties in narcolepsy.
THE MCH -> HISTAMINE CONNECTION: A POSSIBLE MODULATORY CIRCUIT FOR REM SLEEP?

Antoine Adamantidis, Ph.D.

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Rapid-Eye Movement (REM), or paradoxical, sleep correlates with neuronal activity in the brainstem, basal forebrain and lateral hypothalamus (LH). Amongst LH neurons, melanin-concentrating hormone (MCH)-containing neurons are active during sleep, however, their action on REM sleep remains unknown. Here, we generated Tg(Pmch-Cre) transgenic mice and selectively targeted the expression of activatory (ChETA, SSFO) or silencing (eNpHR3.0, ArchT) optogenetic opsins to LH MCH neurons. We showed that acute activation of MCH neurons at REM onset extended the duration of REM, but not non-REM, sleep. In contrast, silencing of MCH neurons during REM sleep reduced the peak frequency and amplitude of theta rhythm in the hippocampus, without affecting the duration of REM sleep episode. We further found that in vitro optical activation of MCH terminals induced fast GABAA-mediated inhibitory post-synaptic currents (IPSCs) in wake-promoting histaminergic neurons of the tuberomammillary nuclei (TMN), while in vivo circuit mapping revealed that TMN neurons accounted for acute MCH neuron extension of REM sleep. Collectively, these results suggest that activation of MCH neurons maintains REM sleep, possibly through a distributed inhibition of arousal circuits in the mammalian brain.
Histamine neurons are exclusively located in the posterior hypothalamus, and project their fibers to almost all regions of the human brain. Although a significant amount of research has been done to clarify the functions of the histaminergic neuron system in animals, a few studies have been reported on the roles of this system in the human brain. In the past studies, the functions of histamine neurons have been clarified using different methods, such as classical pharmacological experiments, histamine-related gene knockout mice and autopsied human brain studies. The histaminergic neuron system is known to modulate wakefulness, the sleep–wake cycle, appetite control, learning, memory and emotion. Histamine neurons have a dual effect on the CNS, with both stimulatory and suppressive actions. As a stimulator, neuronal histamine is one of the most important systems that stimulate and maintain wakefulness. Brain histamine also functions as a suppressor in bioprotection against various noxious and unfavorable stimuli of convulsion, drug sensitization, denervation supersensitivity, ischemic lesions and stress susceptibility. Modern brain imaging techniques including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have now made it possible to study the neural sites and molecular mechanisms underlying crossmodal processing in the human brain. This talk will summarize the neuroimaging studies in humans focusing on the histaminergic neuron system. The molecular and functional neuroimaging studies are also a powerful tool for drug development in this millennium.
HISTAMINE - DOPAMINE INTERACTIONS IN HEALTH AND DISEASE

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Recognition of histamine (HA) as a neurotransmitter came late, long after dopamine (DA). Histaminergic neurons maintain waking, cortical arousal and consciousness while its nearby partners in the hypothalamus, the orexins / hypocretins (Hcrt) organize the motor aspects of vigilance [1]. Surprisingly, neither HA- nor Hcrt-deficient mice suffer from major impairments of waking. Dopaminergic nuclei forming the motor- (A9), reward- (A10) and neuroendocrine- (A11, A12) networks do not display a correlation of neuronal activity with waking. Nevertheless, mice depleted of dopamine neither sleep nor move, and dopamine 2 receptor (D_2R) activation induces REM sleep [2]. In Parkinson`s Disease (PD, loss of A9) D_2R ligands increase sleep attacks [3], which can be treated by blockade of the histamine 3 receptor (H_3R). Histamine does not affect A9 or A10 DA neurons, but excites surrounding GABAergic neurons through the H_1R [4]. Moreover, histamine (H_3R) reduces the release of glutamate, amines and GABA in the striatum [5]. A pedigree with histamine deficiency was discovered suffering from Tourette`s syndrome [6], a disease resulting from dopaminergic dysregulation within cortical-striatal-thalamic circuitry. Interplay between dopaminergic and histaminergic neurotransmissions is studied in transgenic mouse lines (DAT (dopamine transporter)-Cre , HDC-Cre and HDC-/-) in posterior hypothalamus and striatum. A role of HA neurons as most sensitive targets for the effects of L-Dopa therapy in PD is suggested [7]. The cross-talk between HA and DA pathways and receptors opens new avenues in physiology, pathophysiology and therapy.


Dopamine-histamine cross-talk at the level of the tuberomamillary nucleus (TMN). a. Red fluorescent protein (Tmt) under DA transporter (DAT) promoter and immunostaining for HA in green in posterior hypothalamus. b. Typical firing patterns of DA and HA neurons. DA neuron is inhibited, whereas HA neuron is activated by D2/3R ligand quinpirole. c. Connectivity between DA and HA cells in TMNv and DAergic input to TMNv in blue (endogeneous source of L-Dopa). DA and HA neurons express dopa decarboxylase and synthesize dopamine.
NON-PROFESSIONAL HISTAMINE PRODUCING CELLS AND H\textsubscript{4}R IN IMMUNE REACTIONS IN GENERAL AND IN THE PATHOGENESIS OF SJÖGREN’S SYNDROME IN PARTICULAR

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53-kD HDC\textsuperscript{*} (histidine decarboxylase) mast cells (MCs) are professional histamine (HA) producing cells producing HA to storage granules. Their stimulus-triggered burst release produces rather local hot spots and an alarming “on-state” with high but transient ~50 μM HA spikes, able to stimulate low-affinity H\textsubscript{1}R (pK\textsubscript{i} 4.2) and H\textsubscript{2}R (pK\textsubscript{i} 4.3). Homeostatic HA concentrations are soon re-established by dilution, DAO (diamine oxidase)-mediated extracellular degradation and intracellular uptake via organic cation transporters OCT2/3 (SLC22A2/3), followed by reuptake to granules via vesicular monoamine transporter 2 in MCs and cytosolic degradation by HNMT (histamine-N-methyl-transferase) in non-professional HA producing cells. MC number in autoimmune epithelitis/Sjögren’s syndrome (SS) correlates positively with lymphocyte focus score (p<0.01).

In between bursts, 74-kD HDC\textsuperscript{*} salivary epithelial cells produce at a 100-1000-fold lower rate 5-100 nM HA to cytoplasm, from where it is continuously and passively during “off-state” flows through OCT2/3 from acinar and ductal cells into extracellular space, respectively. Salivary epithelial cells do not produce HA degrading DAO, which protects released HA from degradation. Low nanomolar and probably rather universal (rather than topically limited) HA concentrations target the high affinity H\textsubscript{3}R (pK\textsubscript{i} 8.0) and H\textsubscript{4}R (pK\textsubscript{i} 8.3) on myoepithelial and acinar/ductal cells, respectively. This fine-tunes the ~50% intrinsic activity of H\textsubscript{4}R, which might play an anti-apoptotic role and help to maintain immunological self-tolerance.

If mast cell degranulation ensues, the HA-sensitive H\textsubscript{4}R is probably down-regulated, the direction of flow of HA through the “equilibrative uniporters” changes from HA release to HA uptake, followed by intracellular HNMT-mediated degradation in non-professional HA producing cells. Badly down-regulated OCT3 in SS makes the acinar cells unable for HA-mediated homeostatic self- and heterostimulation during the “off-state”, which effect is accentuated by the lack of H\textsubscript{4}R in SS. If these defects are cell autonomous and immune-inflammatory independent, they may further lead to abnormal apo/pyroptosis of salivary epithelial cells, revelation of cryptic, immunogenic antigen epitopes instead of the dominant tolerance-maintaining ones, with consequences for the migratory dendritic cell (DC), DC-mediated antigen presentation and T- and B-cell mediated autoimmune responses. Thus, non-professional HA producing cells may actually professionally maintain the homeostatic fine-tuning of the high-affinity HA receptors. They also participate in down-regulation of the abrupt and violent but short-lasting HA bursts. The relationship between these two strata, low HA/high-affinity receptors and high HA/low-affinity receptors, is incompletely known regarding bidirectional interactions, constitutive and regulated receptor activity, receptor internalization and nuclear histamine.
Oral presentations
ROLE OF THE HISTAMINERGIC SYSTEM IN SHORT-TERM AND LONG-TERM MEMORY OF INHIBITORY ACTIVE AVOIDANCE (IAA)


Short-term and long-term memories involve distinct mechanisms (1). Here we report that brain histamine depletion impairs long- but not short-term memory of IAA. To deplete histamine, we infused into the lateral ventricle of Wistar rats (b.w. 300-340 g) alpha-fluoromethylhistidine (aFMH, 5µg/5µl), a suicide inhibitor of histidine decarboxylase. 24 hrs later rats were placed on a platform facing a grid of stainless steel bars. Once rats stepped down onto the grid, they received an electric shock (0.5mA x 2sec). Rats were re-tested at 2 and 24 hrs after training to assess whether they had retained the shock memory by measuring the step-down latency. When re-tested 2 hrs after training, aFMH-treated and saline-treated rats showed no significantly different step-down latencies (190±35 vs 170±41 sec, n=8, respectively). Conversely, when retested 24 hrs after training, saline-treated rats showed a significantly longer step-down latency compared to aFMH-treated rats (263±16 sec, n=12 vs 37±7 sec, n=15, P<0.0001). Administration of histamine (1µg/1µl) into the basolateral amygdala (BLA) or CA1 hippocampal region immediately after training fully reverted the effect of aFMH infusion (BLA: aFMH + saline: 57±12 sec, n=12 vs aFMH + histamine: 268±15 sec, n=11, P<0.001; CA1: aFMH + saline: 31±7 sec, n=12 vs aFMH + histamine: 246±23 sec, n=10, P<0.001). However, when histamine was given 110 min after training, aFMH effect was reverted only by intra-CA1 administration (aFMH + saline: 45±8 sec, n=8 vs aFMH + histamine: 256±30 sec, n=10, P<0.0001), and not by intra-BLA administration (aFMH + saline: 61±15 sec, n=10 vs aFMH + histamine: 64±16 sec, n=9, ns). We draw 3 conclusions: 1) histamine seems crucial for the long- but not the short-term memory processes of IAA; 2) in the BLA, histamine is involved only in the very early steps of long-term memory formation; 3) histamine in CA1 acts in long-term memory formation independently from BLA.

THE ROLE OF THE HISTAMINERGIC AND OREXINERGIC SYSTEMS IN ANTICIPATORY WAKEFULNESS

K. Ouk *, C. Zhao *, C. Buda, C. Anaclet, Y. Zhao, H. Ohtsu, M. Yanagisawa, JS. Lin

Characterized by increased arousal/wakefulness and enhanced mental and behavioral activity, anticipatory behavior is a vital biological function allowing the organism to foresee diverse internal and external events and to get ready to respond to them. Histamine (HA) and orexins (Ox) are two major wake-promoting systems located in the posterior hypothalamus. Using knockout mouse models lacking HA (HDC-/-) or Ox (Ox-/-) or both Ox and HA (HO-/-), the present study assesses the role of HA and Ox in maintaining wakefulness (W) associated with anticipation entrained by a restricted feeding schedule.

We found that wild type (WT) mice subjected to a predictable restricted food access (11a.m.-5p.m., instead of ad libitum) presented, before and during feeding time, a behavioral activation and a highly significant increase in W characterized notably by a total waking of 70±9 min before the mealtime at 11 a.m.. This anticipatory W was reduced to 42±6 (p<0.05), 14±1 (p<0.01) and 8±4 (p<0.01) min respectively in HDC-/-, Ox-/- and HO-/- mice. Moreover, Ox-/-mice presented a deficit of W enhancement during feeding. When the scheduled food access deliberately was delayed for 20 or 60 min, WT mice maintained a total waking state whereas slow wave sleep, paradoxical sleep (PS) and even direct transition from W to PS (SOREM) occurred during the waiting period in Ox-/-mice (13% of time spent in sleep during 60 min, e.g.) and to a greater extent in HO-/- mice (28 % of time spent in sleep during 60 min, e.g.). Finally, in agreement with a severe anticipatory W deficit seen in HO-/- mice, administration of α-FMH, a specific inhibitor of HA synthesis in Ox-/- mice significantly decreased the already impaired anticipatory W (22±4 vs 8±2 min, p<0.01).

These results support an important role of HA and Ox in maintaining anticipatory W and suggest that Ox and HA act on complement and synergy to ensure anticipatory behavior.

* Equal contribution

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A TRANSCRIPTOMIC AND GENETIC APPROACH TO IDENTIFY MOLECULAR MECHANISMS UNDERLYING WAKEFULNESS

L. Seugnet, C. Anaclet, M. Perier, H. Akaoka, J.S. Lin

Using KO mouse models lacking either histamine (HA, Hdc KO) or orexins (Ox KO), we have previously shown that HA and Ox act distinctly and synergistically in terms of wake control. Importantly, inactivating both HA and Ox results in more defects than the elimination of either system alone, revealing synergistic interactions. The significant wake deficits of the Hdc and Ox double KO (HO KO) mouse, notably somnolence, hypersomnia and EEG deficiency, make it a particularly appropriate model for the identification of new molecular and cellular pathways involved in wake control. To achieve this goal, we have used whole genome profiling to compare cortical gene expression between HO KO mice and their wild type littermates. We found over 200 genes differentially expressed between the two genotypes and notably 1) an increase in cholinergic muscarinic receptors, confirming our own unpublished data suggesting a compensatory over activation of the cholinergic system in HA deficient models. 2) an over-expression of the 5HT-5B receptor, which is thought to function as an auto-receptor co-expressed with the serotonin transporter 3) an up-regulation of the non-coding truncated hdc gene, suggesting the existence of a negative feed-back loop regulating the expression of hdc, potentially mediated by histamine H3 receptor activity. The vast majority of the other differentially expressed genes in HO KO mice have not been previously linked to sleep and waking. Because a thorough screening of these genes is challenging in mammalian systems, we use the Drosophila model which is well suited for this experimental approach. Sleep in Drosophila exhibits many key similarities with mammalian sleep, and can be monitored in thousands of individual animals by automated locomotion detection systems. Drosophila genes can be activated or invalidated in a stage and tissue specific way using the Gal4-UAS system. Initial data obtained in this functional screen will be presented.
ANXIOLYTIC- AND ANTIDEPRESSANT-LIKE ACTIVITIES OF THE NOVEL AND POTENT TRIAZOLE H\textsubscript{3} RECEPTOR ANTAGONIST ST-1283

B. Sadek\textsuperscript{1*}, A. Bahi\textsuperscript{2}, S. J. Schwed\textsuperscript{3,4}, M. Walter\textsuperscript{3}, H. Stark\textsuperscript{4}

Previous studies have suggested a potential linkage of histamine H\textsubscript{3} receptor (H3R) signaling to anxiolytic and antidepressant-like effects. The scope of this study was to investigate the acute effects of the novel selective non-imidazole 1,2,4-triazolyl H\textsubscript{3}R antagonist \textbf{ST-1283} (hH\textsubscript{3}R \textit{Ki} = 0.24 ±0.03 nM) on anxiety- and depression-related behaviours in comparison with diazepam and fluoxetine. The effects of \textbf{ST-1283} were evaluated using the elevated plus maze (EPM) test, open field test (OFT), marbles burying test (MBT), tail suspension test (TST), novelty suppressed feeding (NSF) test, and forced swim test (FST) using male C57BL/6 mice. The results showed that, similar to diazepam, \textbf{ST-1283} (7.5 mg/kg) significantly modified all the parameters observed in the EPM test. In addition, \textbf{ST-1283} significantly increased the time spent in the centre of the arena without altering the general motor activity in the OFT. In the same direction, \textbf{ST-1283} reduced the number of buried marbles as well as the time spent digging in the MBT assay. TST and FST showed that, like the recognized antidepressant drug fluoxetine, \textbf{ST-1283} was able to reduce the immobility time. In the NSF test, \textbf{ST-1283} treatment decreased latency to feed with no effect on food intake in the home cage. More importantly, pre-treatment with the H\textsubscript{3}R agonist R-\alpha-methylhistamine abrogated the anxiolytic and the antidepressant effects of \textbf{ST-1283}. The present series of studies demonstrate novel effects of the newly synthesized H\textsubscript{3}R antagonist \textbf{ST-1283} in number of preclinical models of psychiatric disorders and highlight the histaminergic system as therapeutic target for the treatment of anxiety- and depression-related disorders.

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HISTAMINE H₄ RECEPTORS ARE NOT INVOLVED IN ANXIETY BEHAVIOURAL RESPONSES

P.L. Chazot⁴, R.M. Abuhamdah¹ and A Ennaceur²

We provided the first evidence for functional H₄ histamine receptors on murine central cortical neurons. Expression was detected in selective cortical laminae, the posterior thalamus, and selective subfields of the hippocampus [1]. There is little or nothing known related to the central function of the H₄ receptor. A recent study has suggested that the H₄R has a role to play in anxiety behaviours, but the study used a test which is poorly validated for this behavioural response [2].

In this present study, we investigated whether JNJ7777120, a selective centrally-active H₄R antagonist has any anxiety behavioural effects on BALB/c (high anxiety strain) and CD-1 mice (low anxiety strain), using our novel elevated platform with steep slopes (EPSS) open space anxiety test. Four groups of 3 month BALB/c or CD-1 mice (n = 8) were injected with saline, 5, 10 and 20 mg/kg i.p (effective analgesic doses), JNJ7777120, respectively, 30 minutes prior to exposure to an elevated platform with steep slopes for a single 12 minute session as described in [3]. In contrast to diazepam, neither saline nor JNJ7777120 at all doses tested, prompted any entries onto slopes in the Balb/c mice (no anxiolytic effect). Furthermore, there was no difference between saline and JNJ7777120 at all doses tested in terms of number of slope entries and time spent on the slope (no anxiolytic or anxiogenic effect). However, JNJ7777120 increased motor exploratory activity and number of entries into areas adjacent to slopes, with a concomitant reduction in entries and time spent in central area of the platform, indicating an increased attention response. Overall, these results suggest that the H₄R has no role to play in the anxiety behavioural response.


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GLUTAMATERGIC EXITATION OF HISTAMINERGIC NEURONS.

H.L. Haas, R. De Luca, O.A. Sergeeva

Hypothalamic wake-on neurons, containing histamine (HA) or orexin/hypocretin (Hcrt) fire during waking and support cortical arousal. Optogenetic stimulation of Hcrt axons causes AMPA-receptor-mediated excitation of some mouse HA neurons [1]. We analysed expression and action of glutamate receptors in rat HA neurons by single-cell RT-PCR. The mRNAs encoding for the AMPA-R subunits GluA2 is expressed in 100%, GluA1 in 75% and GluA4 in 56% of rat HA neurons; GluA3 transcripts were not detected [2]. Flip GluA splice variants (potentiated by cyclothiazide) prevail over flop. The fastest desensitization of glutamate (1mM) response is observed in HA neurons expressing GluA4 flop. NMDA receptors (GluN1, GluN2A, GluN2B and GluN2C) are variably expressed in HA neurons, respond to glutamate 10-200µM and can be modulated by steroids [3]. Virtually all rat HA neurons express mGluR1, only 78% mGluR5 [4]. We described an unusually high sensitivity of rat HA neurons to the type I metabotropic receptor agonist DHPG [5]: 3-fold firing in response to 0.5µM. This sensitivity is much higher than in other brain regions, e.g. 100µM in [6]. In mice developmental downregulation of mGluR5 transcripts (but not of mGluR1) is observed. mGluR1-R are coupled to PLC, like several other arousal transmitters signalling to HA neurons and activate TRPC type cation channels. Thus, different types of glutamate receptors mediate HA neuron responses to synaptic inputs (AMPA) or to ambient glutamate (NMDA, mGluR1). The latter is enhanced in response to hypercapnia / hypoxia or ADP [4,5] and serves the homeostatic regulation of neuronal activity.


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**DOPAMINERGIC REGULATION OF THE BRAIN HISTAMINERGIC AND HYPOCRETIN SYSTEMS IN ZEBRAFISH**

*P. Panula, S. Semenova, M. Sundvik, S. Rozov, Y.-C. Chen*

Histamine regulates the number of developing hypocretin neurons in zebrafish, and in narcoleptic patients the number of histidine decarboxylase-immunoreactive (hdc-ir) neurons is increased. In Parkinson’s disease neuronal histamine is increased in affected brain regions, which suggests association between brain dopamine and histamine. This study addressed the role of dopamine in regulation of the brain histaminergic and hypocretin systems. We inhibited both zebrafish tyrosine hydroxylases (th) using morpholino-oligonucleotides in fertilized eggs, counted neurons expressing hdc or hypocretin mRNA and those immunoreactive for histamine. Amine levels were determined using HPLC. Both TH forms contributed to dopamine synthesis. Translation inhibition with morpholino-oligonucleotides of th2, which caused a decrease in dopamine levels, increased the number of hdc-expressing and histamine-immunoreactive neurons significantly in 5-day-old fish larvae. Administration of L-DOPA or dopamine receptor agonists quinpirole or SKF38393 decreased the number of histamine neurons. Similarly, translation inhibition of th2 increased the number of hypocretin neurons in 5-day-old larvae. Interestingly, translation inhibition of th1 which also decreased dopamine levels, had opposite effects: the number of histamine and hypocretin neurons was lower than that in control larvae. We propose that dopamine originating from th2 expressing neurons in the immediate vicinity of the histaminergic neurons has a paracrine regulatory role on terminal differentiation the developing histaminergic neurons, which in turn regulate the developing hypocretin neurons. The role of dopamine derived from th1, which is expressed earlier than th2, may be on proliferation of histamine neuron precursors. The results support the concept that neurotransmitter systems regulate early targets through G protein-coupled receptor mechanisms.

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L-histidine is one of the essential amino acids for humans and plays a critical role as a component of proteins. Histidine is also important as a precursor of histamine. Brain histamine is synthesized from histidine in the presence of histidine decarboxylase expressed in histamine neurons. Although dietary histidine is supposed to be necessary as a precursor of brain histamine, the importance of histidine intake for normal histaminergic nervous system remains unclear. In the present study, we aimed to elucidate the effect of low histidine diet on brain histamine content and behaviours of C57BL/6 mice.

First, we measured histamine and 1-methylhistamine contents in the brains of normal diet-fed mice and low histidine diet-fed (LHD) mice by HPLC. Histamine and 1-methylhistamine contents were significantly lower in LHD mice. However, other monoamines and their metabolites were not changed in LHD mice. In vivo microdialysis assays revealed that histamine release from hypothalamus was decreased in LHD mice. Open field test revealed that LHD mice spent longer time in the peripheral zone. In the light/dark box test, LHD mice spent shorter time in the illuminated space. These results suggested that LHD mice showed anxiety-like behaviours. However, locomotive activity and social interaction were not different between the two groups.

Our present study demonstrated that insufficient intake of histidine reduced the brain histamine content leading to anxiety-like behaviors in mice. Dietary histidine might be essential for a healthy histaminergic nervous system.

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THE INHIBITORY EFFECT OF HISTAMINE IN MOUSE PRIMARY MICROGLIA


Microglia, the brain immune cells, have important roles in the central nervous system (CNS). Microglia migrate toward chemoattractants, engulf bacteria and abnormal proteins, and secrete various cytokines to maintain brain homeostasis. Although previous reports suggested that histamine could regulate intracellular signalling of microglia, the involvement of histamine in microglial functions, such as chemotaxis, phagocytosis and cytokine secretion, remains unclear.

First, we isolated primary microglia from neonatal mouse brains and confirmed that at least 97% cells were CD11b (microglial marker protein)-positive by immunocytochemistry. RT-PCR assays revealed that histamine H3 receptor (H3R) and histidine decarboxylase were expressed in mouse primary microglia. Histamine and imetit, an H3R agonist, inhibited the increase in intracellular Ca\(^{2+}\) concentration induced by ATP stimulation. Forskolin-induced cAMP accumulation was also inhibited by histamine and imetit. These effects of histamine on second messengers in microglia suggested H3R activation by histamine could regulate microglial functions. Indeed, histamine and imetit inhibited microglial phagocytosis, chemotaxis and LPS-induced cytokine secretion (TNFα and Prostaglandin E2 secretion) in a dose-dependent manner. We also confirmed that microglia secreted histamine in the presence of LPS.

In this study, we revealed that H3R expressed in primary mouse microglia inhibited phagocytosis, chemotaxis and cytokines secretion, and activated microglia secreted histamine. These findings suggested that H3R in microglia might regulate microglial functions in an autocrine and/or paracrine manner in CNS.

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HYPOCRETIN/OREXIN NEURONS CONTRIBUTE TO HIPPOCAMPUS-DEPENDENT SOCIAL MEMORY AND SYNAPTIC PLASTICITY IN MICE

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Hypocretin/orexin (Hcrt) and histamine (HA) neurons in the lateral posterior hypothalamus project throughout the brain, including to the hippocampus, where the respective receptors are widely expressed. Hcrt and HA neurons activate these targets to orchestrate global arousal state, wake-sleep architecture, energy homeostasis, stress adaptation, and reward behaviors. Hcrt is implicated in cognitive functions and social interaction. We tested the hypothesis that Hcrt neurons are critical to social interaction, particularly social memory, using neurobehavioral assessment and electrophysiological approaches. The "two-enclosure homecage test" devices and procedure were used to test sociability, preference for social novelty and social memory. A direct contact social test was conducted to corroborate the findings. Orexin/ataxin-3-transgenic (AT) mice, in which Hcrt neurons degenerate by 3 months of age, displayed normal sociability and social novelty with respect to their wild-type (WT) littermates. However, AT mice displayed deficits in long-term social memory. Nasal administration of exogenous Hcrt-1 restored social memory to an extent in AT mice. Hippocampal slices taken from AT mice exhibited decreased paired-pulse facilitation and magnitude of long-term potentiation, but normal basal synaptic neurotransmission in the CA1 area compared to WT slices. AT hippocampi had lower levels of phosphorylated cAMP response element-binding protein (pCREB), an activity-dependent transcription factor important for synaptic plasticity and long-term memory storage. Our studies demonstrate a role for Hcrt neurons in the consolidation of social recognition memory, in part through enhancements of hippocampal synaptic plasticity and pCREB. HA neurons are excited by Hcrt neurons and support this action by HA2R-mediated increase of cAMP in the hippocampus.


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THE HISTAMINE H₄ RECEPTOR IS FUNCTIONALLY EXPRESSED ON RAT DORSAL ROOT GANGLIA NEURON AND GLIA SUBPOPULATIONS

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We have previously reported evidence (using double labelling approaches) that histamine H₄Rs are expressed on both C-fibres (substance P +ve cells) and on putative Aδ fibres at the level of the skin and DRGs (Katebe et al., 2012), which is contrary to the H₃ receptor, which we have previously showed to be present only on subsets of Aδ fibres (Cannon, Chazot et al., 2007).

A model system is required to study the functional and pharmacological properties of neuronal H₄R. Using isolated primary rat DRG neurons, histamine and VUF8430 (selective H₄R agonist) were found to stimulate a rise in intracellular [Ca²⁺]. Sensitivity to 50 mM KCl identified neurons. 1 µM of VUF8430 produced a reversible rise of free intracellular calcium in a subset of cultured primary rat DRG neurons. While a population of neurons appeared to be sensitive, most of the individual VUF8430-sensitive neurons appeared not to be sensitive to capsaicin. VUF8430 displayed a mean EC₅₀ = 4.75 x 10⁻⁸M, and this signal was suppressed by 1 µM JNJ7777120 (selective H₄R antagonist). Based on insensitivity to 50 mM KCl, we identified, for the first time, a population of individual functional H₄R-containing rat DRG associated glial cells.


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ATTENUATION OF ITCH SENSATION IN MICE BY INHIBITION OF THE MAMMALIAN TARGET OF RAPAMYCIN COMPLEX 1 (mTORC1) SIGNALING PATHWAY

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Our earlier studies indicated that inhibition of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway reduced punctate mechanical and cold hypersensitivity in neuropathic pain and, therefore, offered a new approach to chronic pain control, mainly by attenuation of sensitivity of A-nociceptors. In the present study, we extend the list of functions mediated by mTOR-positive primary afferents and investigated their involvement in itch signaling. In adult male C57BL/6J mice bouts of scratching induced by the histamine-dependent pruritogenic compound 48/80 (100 μg) as well as histamine-independent pruritogens, chloroquine (200 μg) and SLIGRL-NH2 (100 μg) injected intradermally were significantly reduced by local (i.d., 12.5nmol) or systemic (i.p., 25kg/kg) pre-treatment with CCI-779 (when injected 6 h before pruritogens). Behavioral observations after systemic pre-treatment with metformin (i.p., 200mg/kg, 4 h before pruritogens) showed that while the response to non-histaminergic stimuli was reduced, scratching to compound 48/80 was not modified by this drug. Observed effects were mediated by mTORC1 signaling pathway as treatment with CCI-779 and metformin at doses effective in reducing of itch-related behaviour, blocked the activity of mTORC1 downstream targets in the spinal cord and dorsal roots (immunoblotting). In addition, we examined the co-localization of P-mTOR with gastrin-releasing peptide (GRP), a marker for some itch-sensitive primary afferents, and found that P-mTOR was co-expressed in less than 5% of GRP-positive fibers in mouse skin (immunohistochemistry). Taken together, our data emphasize the role that P-mTOR positive A-fibers may play in itch signaling and underline the importance of the mTORC1 pathway in the regulation of primary afferent functions such as pain and itch. The actions of the anti-diabetic drug metformin in ameliorating non-histamine mediated itch also suggest a new therapeutic route for the control of this category of pruritis.

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NEWS IN THE RELATIONSHIP BETWEEN HISTAMINE AND BASOPHILS

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Up to recently, basophils were merely viewed as cells capable of storing histamine inside granules from which it could be massively released in response to degranulating agents. However, during the last decade, a wealth of data in the murine system has provided evidence for a more complex relationship between histamine and basophils.

On the one hand it is now clear that various cytokines, such as IL-3, GM-CSF and IL-33, induce histamine production by increasing transcription of the histidine decarboxylase gene (the histamine-forming enzyme), while it has been established more recently that even classical FcεRI crosslinking results in histamine synthesis by a calcineurin-dependent pathway, leading to a 15- to 20-fold increase of the initial histamine content within 20 h.

On the other hand, histamine can in turn modulate basophil functions, more particularly cytokine (IL-4, IL-6 and IL-13) production as well as endogenous histamine synthesis during allergic diseases, through a transport system mediated by Organic Cation Transporter 3 (OCT3).

Lastly, new data suggest that histamine can also influence the interactions between basophils and extracellular matrix taking place in the bone marrow where these cells are located exclusively in type-1 collagen-enriched niches that contain likewise laminin and fibronectin.

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SPECIFIC SENSITIZATION MECHANISM IN MOUSE NICKEL ALLERGY AND THE ROLE OF HISTAMINE IN THIS MODEL

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Millions of people are suffering from metal allergy in the world and their chief symptoms are comprised of skin heat sensation, flare, swelling and blister. The metal is ionized in the body fluid and the ionized metal is absorbed through skin or digestive tract to act as haptens to sensitize the patients. The metal allergy is generally classified as type IV allergic reaction. The metal ion binds to antigen-presenting cells—Langerhans cells and dendritic cells in the skin—and they enter the nearby lymph nodes. These “primed” antigen-presenting cells stimulate naïve T cells to become antigen-specific T cells which enter the systemic blood flow (sensitization phase). Once the same metal ion enters the body it binds to specific protein and acts as an antigen and binds to the antigen-specific T cells to produce skin inflammation by orchestrating neutrophils, macrophage and skin cells (elicitation phase). Lately Toll-like receptor (TLR)4 was proved to be a crucial receptor in human nickel allergy since nickel ion binds to MHC receptor as well as TLR4 and produces signal transduction in antigen-presenting cells to transcribe IRF3 and NF-kB (Schmit et al. Nature Immunol 2010). In contrast to human sensitization mechanism, adjuvant molecule (ex LPS) is necessary to sensitize the mice in nickel allergy. Histamine is reported to contribute to skin thickening reaction in nickel allergy model (Ohtsu et al. Clin Exp Allergy 2007). In that report, however, the cell source of histamine is not investigated because of technical difficulties. Lately, we generated histidine-decarboxylase reporter mice by using bacterial artificial chromosome (BAC). In these mice the emission of fluorescence was observed in TM nucleus in posterior hypothalamus and enterochromaffin-like (ECL) cells in the gastric wall. We observe and characterize the cell surface antigens in fluorescence emitting cells and elucidate the role of histamine producing cells in nickel skin allergy in mice.

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DIFFERENTIAL EFFECTS OF HISTAMINE H₁R AND H₂R VERSUS H₄R ON THYMIC STROMAL LYMPHOPOIETIN (TSLP) BY HUMAN KERATINOCYTES

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Thymic stromal lymphopoietin (TSLP) is a cytokine that plays an important role in inflammatory skin diseases by the induction of proinflammatory cytokines and migration of inflammatory cells, fostering a Th2-inflammatory milieu. It is produced mainly by stromal cells such as fibroblasts, epithelial cells and keratinocytes (KC). Since histamine and TSLP are increased in lesions of inflammatory skin diseases, we investigated a potential effect of histamine and TSLP production by human KC. Human KC were derived from outer hair sheets of donors without skin disease, with atopic dermatitis or psoriasis and stimulated *in vitro* with poly-IC (a known trigger of TSLP), histamine, 2-pyridylethylamine (H₁R agonist), amthamine (H₂R agonist) and ST-1006 (H₄R agonist). TSLP was measured in cell culture supernatants by ELISA. Poly-IC stimulation, but not histamine or HR-agonists alone, resulted in a significant increased of TSLP. Preincubation of cells with histamine and H₁R or H₂R agonists before polyIC stimulation resulted in significant increases of TSLP production, whereas the H₄R agonist yielded a downregulation of TSLP. These effects were seen in cells derived from donors without and with inflammatory skin diseases.

Taken together, our results indicate that the H₄R might alleviate a Th2 milieu and might have anti-inflammatory properties, whereas H₁R- and H₂R-stimulation might produce inflammation.

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IMPROVEMENT OF SYMPTOMS WITH CORRELATIVE SUPPRESSION OF ALLERGIC DISEASE-SENSITIVE GENE EXPRESSION

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Stimulation of histamine H1 receptors (H1R) activated H1R gene expression in HeLa cells, and induced H1R up-regulation. H1R mRNA and H1R were also elevated in nasal hypersensitivity model rats after provocation, and pre-treatment of antihistamines suppressed the elevation. Elevation of H1R mRNA level in the nasal mucosa of pollinosis patients and suppression of the elevation by pre-treatment of antihistamines were studied. Correlation between nasal symptoms and mRNA levels of H1R, histidine decarboxylase (HDC) and interleukin-5 (IL-5) were also studied. Among 25 pollinosis patients 8 patients were pre-treated with antihistamines for 18 days prior to the onset of pollen season. Symptoms were scored by the levels of sneezing and rhinorrhea. Samples for mRNA determination were biopsied from nasal mucosa, and mRNA was determined by real time-PCR. Pre-treatment of antihistamines showed the improvement of symptoms and stronger suppression of H1R mRNA level. Positive correlation between symptoms and mRNA levels of H1R, HDC and IL-5 was observed. Improvement of symptoms was obtained by the pre-treatment of antihistamines with correlative suppression of H1R gene expression. Genes of H1R, HDC and IL-5 were suggested to form a group of allergic disease-sensitive genes.

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BIASED AGONISM AT THE HISTAMINE H₁ RECEPTOR: CHIRAL HISTAPRODIFEN DERIVATIVES SHOW FUNCTIONAL SELECTIVITY FOR \(\beta\)-ARRESTIN RECRUITMENT

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According to the classical model seven transmembrane domain (7TM) receptors activate heterotrimeric G-proteins, leading to stimulation or inhibition of downstream signaling cascades. Subsequent \(\beta\)-arrestin binding to the receptor prevents further G-protein coupling and triggers receptor internalization. According to this model, the receptor serves as a simple on-off switch, which interacts with the G-protein and \(\beta\)-arrestin in a balanced manner. However, an increasing number of ligands have been shown to exhibit functional selectivity at their receptors, preferentially activating either G-protein or \(\beta\)-arrestin in a biased manner. This is of particular interest as \(\beta\)-arrestin was demonstrated to serve as a scaffold for diverse signaling molecules resulting in physiological responses distinct from G-protein mediated pathways.

In this study, we investigated functional selectivity of histamine H₁ receptor (H₁R) ligands. To address G-protein activation, we chose the \([^{32}\text{P}]\) GTPase assay, measuring the rate of hydrolysis of radiolabeled GTP by the Gα subunit. \(\beta\)-Arrestin recruitment was measured by a luciferase complementation assay. We selected 27 H₁R ligands, including 14 known H₁R antagonists as well as a set of agonists such as histamine and several phenylhistamine and histaprodifen derivatives. None of the antagonists produced a significant effect in both the GTPase and the \(\beta\)-arrestin recruitment assay. Among the histaprodifen derivatives, several chiral compounds revealed bias towards \(\beta\)-arrestin activation, behaving as neutral antagonists in the GTPase assay but possessing partial agonist activity in \(\beta\)-arrestin 2 recruitment. Interestingly, in contrast to histamine, those compounds had no efficacy towards \(\beta\)-arrestin 1 recruitment, thus showing bias between the two \(\beta\)-arrestin isoforms.

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H₄ RECEPTOR AGONISTS ARE WELL TOLERATED IN MICE WITH NO ADVERSE HEMATOLOGICAL FINDINGS

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H₄ antagonists have been shown to have anti-inflammatory properties in preclinical models of autoimmune disease, and are currently being evaluated for their therapeutic potential in humans. The functional behaviour of H₄ modulators have been shown to be highly dependent on assay conditions. Thus, it is important to understand any potential toxicological consequences of the full spectrum of H₄ functional behaviour from antagonists to full agonists. A number of H₄ antagonists have been disclosed in the literature, and are valuable tools for interrogating the biology of H₄ signaling. Several H₄ antagonists have demonstrated good preclinical tolerability and advanced into human clinical trials. However, the available data on the tolerability of H₄ agonists is currently limited. Some H₄ agonists have been associated with toxicological outcomes including lymphoid depletion and reduced erythropoiesis. However, whether these effects are compound specific or should be directly attributed to H₄ agonism is unknown and deciphering this would require utilizing H₄ agonists from diverse chemotypes in order to make definitive conclusions.

During our search for new H₄ antagonists, we discovered a series of compounds that demonstrate partial to full agonism at rodent H₄ receptors in vitro and have pharmacokinetic profiles that are suitable for studying the effects of chronic stimulation of the H₄ receptor. In vivo evidence of agonism was demonstrated in a mouse pruritus model, wherein dose-dependent increases in pruritus could be suppressed by the addition of an H₄ antagonist, JNJ-7777120.

To assess the potential toxicological effects of H₄ agonists, three selected compounds were tested in a four day mouse toleration study. There were no significant changes in hematology, clinical chemistry, or histomorphology including bone marrow cellularity for the three compounds. The results of this experiment indicated that no adverse effects in rodents could be attributed to chronic stimulation of the H₄ receptor. JNJ-41952963, JNJ-42814187 and JNJ-52142142 are potent mouse agonists having PK profiles that are suitable for studying H₄ agonists in chronic disease models.

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EFFICACY AND SAFETY OF THE H₄R ANTAGONIST JNJ-39758979 IN A PHASE 2A CLINICAL STUDY IN PATIENTS WITH ATOPIC DERMATITIS


The safety and efficacy of the histamine H₄R antagonist JNJ-39758979 was evaluated in adult Japanese patients with moderate atopic dermatitis. Patients were randomized to receive either 100 mg or 300 mg of JNJ-39758979 or placebo once-daily for 6 weeks. The primary efficacy was assessed at week 6 using Eczema Area and Severity Index (EASI) scores. Pruritus was assessed by patient-reported assessments including Pruritus Categorical Response Scale, Pruritus Numeric Rating Scales, Pruritus Interference Numeric Rating Scale, and Subject’s Global Impressions of Change in Pruritus. Eighty-eight of 105 planned patients were randomized before the study was stopped for safety reasons and 49 patients reached week 6. The efficacy analysis was performed post hoc and patients who failed the study treatment and took other treatment(s) that may affect efficacy evaluation or dropped out had their last observation before failure carried to the primary time point, week 6. Since most patients were withdrawn from the study before the week-6 assessment, missing data were not imputed. The primary endpoint was not met as there were no statistically significant differences in the EASI score at week 6. However, numerical improvements (i.e., decreases) in median EASI score were observed in the 100 mg (-3.7) and 300 mg (-3.0) JNJ-39758979 groups compared to placebo (-1.3). Nominally significant improvements were observed in several of the pruritus assessments. Adverse event rates were comparable between JNJ-39758979 and placebo with the exception of two patients (both receiving JNJ-39758979 300mg) with serious adverse events of neutropenia, leading to premature study discontinuation. This drug-induced agranulocytosis is thought to be related to the generation of reactive metabolites of JNJ-39758979 and not to the mechanism of antagonism of the H₄R. The findings suggest H₄R-antagonism may be beneficial for the treatment of atopic dermatitis and in particular in the control of pruritus.

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In contrast to the mouse and rat H₄R, the human H₄R shows a high degree of constitutive activity. Aiming at more detailed insights into the molecular determinants of ortholog-dependent ligand-receptor interactions, we generated and expressed (Sf9 cells) a series of H₄R mutants to determine radioligand binding and functional data ([³⁵S] GTPγS assay). Apart from F169, which was identified by Lim et al. as a key amino acid for distinct ligand binding affinities at H₄R orthologs, we mutated, based on molecular modeling studies, S179, S330 and R341 to the corresponding amino acids of the rodent H₄Rs, resulting in hH₄R-F169V, hH₄R-S179M/A, hH₄R-F169V+S179M or hH₄R-F169V+S179A, hH₄R-S330R and hH₄R-R341S. Moreover, to study the role of the F168/F169 motif, which is also found in, e.g., the β₂AR, H₃R and the M₂R, we expressed the hH₄R-F168A mutant in Sf9 cells. Whereas changes in ligand affinity and potency were only minor, the constitutive activity of the hH₄R-F169V and the double mutants was significantly reduced compared to the wild-type hH₄R. By contrast, an exchange of S179 by M or A alone did not significantly affect constitutive activity. Strikingly, the double mutants were comparable to the mH₄R and to the rH₄R, which are devoid of constitutive activity. The inverse agonism of thioperamide decreased from the hH₄R via the hH₄R-F169V mutant to the hH₄R-F169V+S179M and hH₄R-F169V+S179A double mutants, respectively. The data for the hH₄R-F168A mutant revealed a major contribution of F168 to ligand binding with a concomitant, up to over 100-fold decrease in ligand potencies and a complete loss of constitutive activity, compared to the wild-type hH₄R. Thioperamide acted as a neutral antagonist and JNJ7777120 turned to partial agonism. The results suggest that, in particular, F168 alone and F169 in concert with S179 favor the switch from the inactive to the active state of the human H₄R.

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TRIAZOLOQUINOXALINES AS POTENT H₄ RECEPTOR LIGANDS


The currently known H₄R ligands contain an indolecarboxamide, quinazoline or pyrimidine scaffold and within these series some promising drug candidates have been identified (e.g. PF-3893787 and JNJ-40279486). Most of these ligands display either a high ClogP or a high pKₐ in combination with a second basic center with a medium pKₐ. It is well known that these properties frequently result in challenges in drug development, limiting the chance of successfully reaching the clinic. We therefore set out to discover a new H₄R ligand series with properties in the low-risk physicochemical area (low ClogP, one basic center with a medium pKₐ). This study presents a new chemical series, the triazoloquinoxalines, which have comparable H₄R affinity, metabolic stability, solubility and lipophilicity as the current most advanced H₄R ligands PF-3893787 and JNJ-40279486. In contrast to the latter compounds, the newly described triazoloquinoxalines are characterized by a single basic center, which in combination with the low lipophilicity brings these compounds in the low-risk physicochemical property area.

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PRECLINICAL CHARACTERIZATION OF THE HISTAMINE H₃ RECEPTOR ANTAGONIST BAVISANT (JNJ-31001074) AND DETERMINATION OF DRUG LEVELS REQUIRED FOR ROBUST TARGET ENGAGEMENT IN HEALTHY VOLUNTEERS.

N. I. Carruthers

We recently described several series of histamine H₃ receptor antagonists together with their preclinical characterization, via *in vivo* microdialysis and *ex vivo* autoradiography, and in animal models predictive of efficacy in diseases of the central nervous system. Subsequently this afforded three clinical candidates that were evaluated in narcolepsy, allergic rhinitis, the treatment of scopolamine induced cognitive impairment and adult ADHD. The background leading to the discovery of Bavisant (JNJ-31001074) a potent and selective histamine H₃ receptor antagonist will be presented emphasizing the use of imaging techniques, including Positron-Emission Tomography (PET) in healthy volunteers, to establish a relationship between drug levels and receptor occupancy.

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DISCOVERY OF NATURAL BASED & CARDIOSAFE LIGANDS OF HUMAN H4R USING COMPUTERIZED TECHNIQUES

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The human Histamine H4 Receptor (hH4R) has attracted much interest as potential drug target for treatment of several inflammatory, allergic and autoimmune disorders, as well as for analgesic activity. None of the hH4R ligands was successful to get into the market up to date and there is still a strong demand for new selective ligands to be developed.

Isolation and identification of bioactive chemicals in wet lab is time consuming and very expensive. In the present study we describe how using in-silico techniques could facilitate achieving such goal. As well, the majority of the currently used drugs are natural products-based compounds or their derivatives and there is an indication that these compounds have more chance to survive the drug development process. Furthermore, natural product-based chemical libraries have drug-like properties more than synthetic compound libraries.

Aiming to increase hit rate for selecting bioactive chemicals in the virtual screening process, we applied sequentially ligands-based models followed by structure-based models. Two ligand-based chemoinformatics techniques, the Intelligent Learning Engine and Iterative Stochastic Elimination approach, were utilized to index natural products for their hH4R antagonism. A virtual high throughput screening on ZINC-natural products database was carried out and we picked ~300 highly indexed as H4R antagonists candidates. The highly indexed natural products selected from the ligands-based study were docked into the hH4R 3D model and re-ranked. Apart of the AutoDock energy and particularly the electrostatic term, the filter of the ability to interact with Asp94 (TM3) and Glu182 (TM5) via hydrogen bonding/ electrostatic interaction was taken into consideration. A model for prediction of cardio-safety was applied on the highly indexed ligands and several natural-based chemicals were selected for further experimental testing. In conclusion, sequential combination of the ligands-based chemoinformatics techniques followed by structure-based bioinformatics techniques has the potential to disclose new biologically active natural saving time and money.

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ANALYSIS OF MOUSE POLYSPECIFIC TRANSPORTERS


Specific histamine transporters have not been found until now, although other monoamines in synaptic cleft are transported through specific transporters such as serotonin transporters and dopamine transporters. Recent studies reveal that histamine can be transported through organic cation transporter (Oct) 2, Oct3 and plasma membrane monoamine transporter (Pmat). These transporters belong to polyspecific monoamine transporters which can transport various monoamines. However, the importance of these transporters for histamine clearance in vivo remains unknown. In the present study, we examined the detailed characteristics of mouse Oct2, Oct3 and Pmat, and their expression levels in 12 mouse brain areas.

We first characterized these transporters by uptake study using CHO cells stably overexpressing mouse Oct2, Oct3 or Pmat. We confirmed that these transporters could transport histamine in a time- and dose-dependent manner. Kinetic analysis revealed that Oct2 had the highest affinity for histamine among them. Transport activities of mouse Oct2 and Oct3 were partly dependent on extracellular Na+/Cl- concentrations and increased in alkaline conditions. On the other hand, the transport activity of mouse Pmat was independent of extracellular Na+/Cl- concentrations and increased in acidic condition.

Next, we performed quantitative RT-PCR to reveal the expression levels of these transporters in each brain area. Mouse Oct2 was highly expressed in olfactory bulb and prefrontal cortex. Oct3 in striatum and hypothalamus, and Pmat in medulla and cerebellum, were also abundantly expressed.

Our study for the first time described the detailed characteristics of mouse Oct2, Oct3 and Pmat. We hope to uncover the importance of these transporters for brain histamine clearance in vivo.

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DIFFERENTIAL SIGNALING PATHWAYS OF THE hH₁R AND THE hH₂R

N. Kagermeier, U. Nordemann, G. Bernhardt, G. König, A. Buschauer

Aiming at functional assays enabling the discrimination between different signaling pathways activated by histamine receptor (HR) stimulation, we genetically engineered HEK293T cells, stably expressing the firefly luciferase under the control of a cAMP response element (CRE), by stable transfection with the hH₁R or the hH₂R, respectively. The transfectants were investigated in a luciferase assay and in a fura-2 calcium assay. As expected, the stimulation of the hH₁R by histamine led to a concentration-dependent increase in intracellular Ca²⁺. Surprisingly, the activation of the hH₂R resulted in a Ca²⁺ signal as well. Interestingly, the activation of the hH₁R induced an increase in the transcription of luciferase, presumably via the activation of Ca²⁺-calmodulin-dependent kinases. In the presence of 1 μM UBO-QIC, a selective Gαq protein inhibitor, the maximal response was reduced by approximately 50%. The expression of luciferase was completely suppressed by the PKA inhibitors Rp-cAMP-S and Rp-8-Br-cAMP-S.

In the luciferase assay, the hH₂R revealed high constitutive activity, probably due to a very high expression level of the receptor (2.5 million receptors per cell). The high basal levels were reduced by adding different concentrations of forskolin, presumably via the activation of ICER (inducible cyclic AMP early repressor). In this setting, histamine amplified the luciferase signal, suggesting that this is not a cAMP-mediated response, but rather a Ca²⁺ induced effect. The Gq inhibitor UBO-QIC reduced the histamine-induced maximal response by approximately 35%.

In summary, the data indicate that, regardless of preferential coupling of hH₁R and hH₂R to Gαq and Gαs, respectively, both receptors are capable of triggering differential signaling pathways, at least in genetically modified cells used for pharmacological characterization of HR ligands.

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SPECIFIC TARGETING OF HUMAN ALLERGIC EFFECTOR CELL FUNCTION USING GOLD-BASED NANOMATERIALS

B.F. Gibb, L.Calzolai, V.V.Sumbayev

Several signal transduction inhibitors prevent the pro-allergic functions of mast cells and basophils but are rarely used systemically due to ubiquitous expression of signalling proteins. However, specific targeting of these cells with such inhibitors could bypass unwanted side effects, also due to the lower concentrations of potentially toxic inhibitors used. We recently reported that gold nanoparticles (AuNPs) can be employed for the non-toxic delivery of signalling inhibitors due to their unique physicochemical properties. Because AuNPs can be conjugated with both anti-allergic drugs and antibodies that specifically recognize mast cells and basophils our aims were to assess the specific targeting of allergic effector cell function using AuNPs conjugated with signal transduction inhibitors. Purified human basophils and LAD2 human mast cells (provided by Kirschenbaum et al, Leuk Res 2003;27:677-82) were used for investigations of AuNPs conjugated to antibodies against CD203c amine-coupled to acidic groups of reduced glutathione (GSH). GSH was also used as a spacer for immobilisation of the calcineurin inhibitor ascomycin on the gold surface. AuNPs alone did not affect basophil histamine release or viability. However, AuNPs conjugated with anti-CD203c specifically bound to basophils and, when conjugated with ascomycin, they inhibited IgE-dependent basophil histamine release at effective concentrations of 5 nM ascomycin (20-fold more potent than ascomycin alone). Similar results were obtained using mast cells and unpurified basophils present in mixed leukocyte preparations, suggesting specific targeting of these cells. Successful targeting of allergic effector cells using gold nanoconjugates indicates that this technology may be useful in anti-allergic therapy for specifically delivering highly effective signalling inhibitors without side-effects. The principle can be applied to various agents (drugs, toxins) and to a variety of experimental and clinical scenarios.

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IMPACT OF ORAL VITAMIN C ON HISTAMINE LEVELS AND SEASICKNESS

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Seasickness is a risk aboard a ship. Histamine is postulated as a causative agent, inversely related to the intake of vitamin C. Persons with mastocytosis experienced improvement of nausea after the intake of vitamin C.

To determine whether vitamin C suppresses nausea in 70 volunteers who spent 20 minutes in a life raft, exposed to one-meter-high waves in an indoor pool. Double-blind placebo-controlled crossover study. Two grams of vitamin C or placebo was taken one hour before exposure. Blood samples were taken one hour before and after exposure to determine histamine, diamine oxidase, tryptase, and vitamin C levels.

Symptom scores were noted on a visual analog scale. On the second day the test persons were asked which day they had felt better.

Seven persons without symptoms were excluded from the analysis. Test persons had less severe symptoms after the intake of vitamin C (p<0.01). Scores on the visual analog scale were in favor of vitamin C, but the difference was not significant.

Twenty-three of 63 persons wished to leave the raft earlier: 17 after the intake of placebo and 6 after the intake of vitamin C (p<0.03). Women (p<0.02) and men below 27 years of age (p<0.02) had less pronounced symptoms after the intake of vitamin C. Histamine (p<0.01) and DAO levels were increased after the intake of vitamin C (p<0.001) and after placebo (n.s.). The fact that the second test day was rated less stressful by most volunteers is indicative of habituation.

Some of the data show that vitamin C is effective in suppressing symptoms of seasickness, particularly in women and men younger than 27 years of age, and is devoid of side effects. Histamine levels were initially increased after the test persons had been exposed to waves.

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HISTAMINE H\textsubscript{3} RECEPTOR LIGANDS RETAINS INTRAOCULAR PRESSURE (IOP)-LOWERING ACTIVITY AND ENHANCES VASCULAR FLOW IN RETINIC ARTERY IN GLAUCOMA MODELS.

C. Lanzi\textsuperscript{1}, L. Lucarini\textsuperscript{1}, N. Mugelli\textsuperscript{1}, A. Pini\textsuperscript{2}, D. Łażewska\textsuperscript{3}, K. Kieć-Kononowicz\textsuperscript{3} and E. Masini\textsuperscript{1}.

Elevated intraocular pressure (IOP) is the major risk factor for the development of glaucoma, therefore the reduction of IOP is usually considered the mainstream of glaucoma therapy. Significant histamine levels has been found in retina, choroid tissue, optic nerves and ocular mast cells. Histamine H\textsubscript{3} receptors control the synthesis and release of histamine. The present work is focused on the evaluation in different models of glaucoma, in albino rabbits, the effects of selective histamine H\textsubscript{3} drugs in reducing IOP and improving ocular vascular perfusion.

Ocular hypertension was obtained by the injection of 0.1 ml of hypertonic saline (0.9\%) into the vitreous or carbomer (0.1\%) in the anterior chamber of New Zealand albino rabbits. IOP was measured using a Tono-Pen. The examinations were performed prior to saline or carbomer injection (baseline), immediately before drug dosing (pre-treatment) and 1, 2, 3 and 4 hour after in the acute saline model and every day for a week in the chronic carbomer model. All the animals underwent Color Doppler Imaging investigation before and at the end of drug treatment. Blood flow velocities were measures for ophthalmic and ciliary artery and the Pourcelot Resistance Index (RI) calculated. Biochemical and morphological changes were also assessed in the aqueous humour and in retinal biopsies.

On average, IOP rose from 18.4±2.0 mmHg at baseline to 38.9±4.0 mmHg after hypertonic saline injection and from 17.7±3.3 to 42.6±3.4 one day post carbomer injection and remained stable thereafter. ABT-230 (1\% solution) a selective histamine H\textsubscript{3} antagonist and DL-76 (0.5-1\% solution), a selective H\textsubscript{3} ligand, lowered IOP at all time points post saline and carbomer injection After repeated administration of the two drugs the Pourcelot RI of ophthalmic artery was significantly reduced and oxidative stress markers decreased.

Histaminergic H\textsubscript{3} ligands could represent an interesting new therapeutic option for the treatment of glaucoma.

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HISTAMINE H₃ RECEPTOR EXPRESSION IN THE HUMAN KIDNEY

E. Veglia¹, C. Grange², A. Pini³, G. Camussi², P.L. Chazot⁴, A.C. Rosa¹

Renal expression of histamine H₁ receptors (H₁R), H₂R, and H₄R has been previously demonstrated; that of H₃R has never been reported. Aim of the present study is to evaluate the expression of functional H₃R in human kidney. Specimens from kidney of 12 patients undergoing elective nephrectomy were processed for immunohistochemical analysis. Moreover, ligand-induced intracellular cAMP production (TR-FRET method) was measured in primary (p) and immortalized (i) tubular epithelial cells (TEC) from the cortex of human specimens.

Our data show a clear H₃R immunoreactivity on tubular epithelium. Consistently, receptor expression (RT-PCR and Western blot) was demonstrated in both iTEC and pTEC, but not in HK-2 cells (proximal tubular cells). In iTEC histamine (3 pM - 10 nM, 30 min) modulates intracellular cAMP levels in a concentration-dependent manner. Ranitidine (10 µM), GSK189254 (1 µM) and JNJ7777120 (10 µM), which are H₂R-, H₃R- and H₄R-selective antagonists respectively, partially and in a differential manner affected the histamine-induced cAMP production when added alone, while blunting it completely when combined together.

In conclusion, our data demonstrate that pTEC express H₂R, H₃R and H₄R. This study provides the first evidence for the presence of H₃R in epithelial cells of human tubules.

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O30

FUNCTIONAL IDENTIFICATION OF THE H₄R IN THE HUMAN KIDNEY

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The expression of histamine receptor H₄R, in the tubules of rat kidney, and its over-expression in diabetic animals, has been recently demonstrated in our laboratory [Rosa et al., 2013]. In order to confirm these findings in the human kidney, we have investigated the expression of H₄R in specimens of healthy human renal cortex and in cultured human renal cells.

Specimens from the kidney of 12 patients underwent elective nephrectomy have been processed for immunohistochemical analysis. These data were complemented by the evaluation of the H₄R expression through immunoblotting and RT-PCR analysis in human immortalized cell lines (podocytes, mesangial cells, and the proximal tubular epithelial cell line HK-2), and in primary cultures of human glomerular and tubular cells from human kidney specimens. Moreover, histamine-triggered cAMP production has been measured by the TR-FRET method to study receptor responsiveness.

The gene and protein expression of the H₄R have been detected in all the cell types tested. The contribution of H₄R to histamine-evoked cAMP production was defined by the selective antagonist JNJ7777120 (10 µM). While partially blunted in some of the cell lines, the histamine response was completely abolished only in the HK-2 cell line.

In conclusion, our data demonstrate that H₄R is expressed by both glomeruli and the proximal tubular cells, thus suggesting a possible involvement of this receptor in renal (patho)physiology which remains to be established.


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HISTAMINE H4 RECEPTOR AGONISTS ARE SYNERGISTIC WITH DOXORUBICIN IN BREAST CANCER

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We have previously demonstrated that the histamine H4 receptor (H4R) is expressed in human breast cancer tissue and cell lines, exhibiting a key role in histamine-mediated biological processes such as cell proliferation, senescence, and apoptosis.

The aim of this study was to investigate whether H4R agonists could synergize the effects of chemotherapy in vitro and in vivo in breast cancer models. For that purpose, the effect of doxorubicin (DOX) combined with H4R agonists on processes associated with cell death and survival, metabolism of ROS and DNA damage was studied in human breast cancer cell lines (MDA-MB-231 and MCF-7). The combination therapy of DOX with H4R agonists was further investigated in vivo in human triple negative breast cancer induced in nude mice with MDA-MB-231 cells.

Results indicate that DOX inhibited clonogenic proliferation in a concentration dependent manner, and the combined treatment of histamine (HA), clozapine or VUF8430 with DOX (1 nM) significantly synergized the inhibitory effect on proliferation, sensitizing DOX-induced cell death. Also, the combination of histamine with DOX enhanced the decrease in the incorporation of BrdU in both cell lines 48 h after treatment [% BrdU positive MDA-MB-231 cells: 50.7±4.2 (control), 40.8±1.3 (DOX), 36.6±1.8 (HA) vs. 30.9±2.7 (DOX+HA), P<0.01). % BrdU positive MCF-7 cells [41.3±1.1 (control), 30.3±2.2 (DOX), 28.8±1.8 (HA) vs. 17.2±1.0 (DOX+HA), P<0.01]). This effect was mimicked by the H4R agonists. H4R ligands potentiated the increase in ROS only in MCF-7 cells. Accordingly, in vivo treatment of MDA-MB-231 tumors with HA enhanced the DOX-induced reduction in growth rate, increasing tumor doubling time (7.5±0.7 in control group; 24.9±1.0 in DOX group, P<0.001; 30.9±1.2 days in DOX+HA group, P<0.001) and decreased mitotic index and tumor cellularity that was replaced with extracellular matrix.

We conclude that H4R agonists enhance the antitumoral effects of DOX in vitro and in mouse xenograft models, suggesting that they could be potential adjuvant agents to improve the therapeutic index of chemotherapy in the treatment of breast cancer.

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HISTAMINE H₃ RECEPTOR-DEPENDENT MEDIATION OF BRAIN DOPAMINERGIC REGULATION IN MICE

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Our previous studies have established that both pharmacological blockade and genetic knockout of the H₃ receptor lead to diminished alcohol reward, consumption and stimulation in mice. However, these studies have not revealed the mechanism behind these phenomena. The aim of this study was to investigate by which mechanism H₃ receptor inhibits alcohol reward and consumption. As the dopaminergic system is a key component of both reward and locomotion, we hypothesized that the interaction between the dopaminergic and histaminergic systems might be responsible for these altered behaviors.

By quantitative radioactive in situ hybridization we analyzed the expression of D₁ and D₂ receptors in the striatum and tyrosine hydroxylase and dopamine transporter mRNA in the ventral tegmental area in WT and H₃ receptor knock-out (H₃R KO) mice, and found no differences between the genotypes. Interestingly, we found that the locomotor response to D₂R agonist quinpirole was altered in H₃R KO mice, while the response to D₁R agonist SKF-38393 was unaltered. By quantitative western blotting, we then studied the effects of SKF-38393 and quinpirole on cell signaling in WT and H₃R KO mice. Mouse brains were removed 20 minutes after drug treatments and the striata were collected as an important area in reward and locomotion. From the striatal homogenates we analyzed the phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK1/2) and cAMP response element binding protein (transcription factor CREB) which are activated e.g. upon G-protein coupled receptor activation and contribute to drug-induced changes in cell signaling. Interestingly, we found that ERK1/2 was phosphorylated after both D₁ and D₂ receptor agonist treatments in the WT mice, but not in the H₃R KO mice. In conclusion, these studies suggest that dopaminergic signaling requires functional H₃ receptors and the lack of ERK1/2 activation in H₃R KO mice might partly explain the behavioral defects seen in these mice.

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Current pharmacological treatment for neuropathic pain is unsatisfactory in many cases due to limited efficacy and side-effects. Further investigation of neuropathic pain pathophysiology is necessary for validating new pharmacological treatments to achieve efficient analgesia. Histamine has been widely implicated in the development of nociception and neuropathic pain. Histamine H_1, H_2 and H_3 receptor ligands have been shown to reduce hyperalgesia following nerve injury, but the role of histamine H_4 receptors (H_4R) has not been fully elucidated. To investigate the role of histamine H_4R in neuropathic pain, the H_4R agonists VUF8430 and ST1006 were used to assess their effects following peripheral nerve injury.

Mice subjected to spared nerve injury (SNI), a model of neuropathic pain, showed a long lasting mechanical (von Frey’s test) and thermal (plantar test) hyperalgesia. I.c.v. administration of both H_4R agonists dose-dependently reversed pain hypersensitivity in the ipsilateral hind limb and, at the highest doses, induced antinociception in the contralateral limb. The increase of pain threshold was completely prevented by the H_4R antagonist JNJ10151984 that was devoid of any effect when administered alone. Mice undergoing treatment with H_4R receptor ligands did not show any side effect or sign of toxicity. To further investigate into the mechanism of action of H_4R agonists, we determined the capability of these compounds to counteract the oxidative damage induced by nerve injury. A significant increase of PARP activity and 8OHdG and decrease of MnSOD was observed in the ipsilateral spinal cord and sciatic nerve of SNI mice. These effects were completely prevented by VUF8430. No effect was detected on the contralateral limb.

This study presents evidence for a thermal and mechanical antinociception by H_4R agonists in a model of neuropathic pain through an antioxidant mechanism. H_4R activation may represent a new therapeutic perspective for neuropathic pain management.

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FUNCTIONAL FRAGMENT SCREENING AT GPCRS: TOWARDS SIGNALLING-BIASED FRAGMENTS.

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Fragment-based drug discovery (FBDD) is a widely acknowledged drug discovery approach to identify new hit molecules at a variety of targets. The lower complexity of small fragments compared to larger drug-like compounds increases the chance of matching interactions with accessible binding sites. We have recently pioneered the application of fragment-based approaches in the field of G protein-coupled receptors (GPCRs) by employing high-concentration fragment screening by radioligand binding displacement and subsequent fragment optimization, which led to various new series of potent histamine H₄ receptor (H₄R) ligands.

GPCRs acquired their name from the ability to activate G proteins to induce intracellular signalling, although it is now known that GPCRs can interact with a variety of intracellular proteins (e.g. β-arrestins can function as signalling scaffolds). Interestingly, GPCR ligands do not always activate these downstream signalling pathways with equal efficacy. This so-called "biased signalling" has been observed for various GPCRs, including H₄R. Intriguingly, the structural and/or molecular basis for ligand efficacy and biased GPCR signalling is still unclear.

In this study, we extended our FBDD approaches to investigate fragments with specific profiles with respect to (biased) functional activity at the histamine GPCR family (i.e. H₁R-H₄R). We therefore complemented our radioligand displacement studies with fragment efficacy measurements in both G-protein activation and β-arrestin2 recruitment. This resulted in the identification of histamine receptor agonists and antagonists, allosteric binders and biased fragments. Importantly, whereas most efficacious fragments target multiple histamine receptors subtypes, also receptor subtype specific fragments were found. Currently running chemogenomic analyses will shed light on the molecular requirements for histamine receptor-specific ligand efficacy, and biased signalling.

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Histamine is a key immunoregulatory mediator in both immediate type hypersensitivity reactions and chronic inflammatory responses. Toll-like receptors (TLR) recognize bacterial ligands and histamine alters TLR signaling cascades, in particular via histamine receptor 2 (H\textsubscript{2}R). However, the influence of histamine on TLR responses in cells from inflammed patients has been poorly described. The aim of this project is to investigate the effects of histamine on TLR-2 stimulated PBMCs isolated from patients suffering from inflammatory bowel disease (IBD).

Forty eight patients diagnosed with IBD (24 with Crohn’s disease and 23 with ulcerative colitis) and 24 healthy volunteers were included in the study. Expression of histamine receptors (H\textsubscript{1}, H\textsubscript{2}, H\textsubscript{4}R), histamine-related enzymes (HDC, HNMT and DAO) as well as TLR-1, TLR-2, TLR-4, TLR-5 and TLR-9 was investigated using REAL-TIME PCR. Cytokine levels were determined in culture supernatants after 24-hour Pam\textsubscript{3}-Cys (TLR-2 ligand) stimulation in the presence or absence of histamine or famotidine (H\textsubscript{2}R antagonist).

Significant differences in H\textsubscript{1}R, H\textsubscript{4}R, TLR-6, TLR-9 and DAO gene expression were observed in patients with IBD compared to healthy volunteers. Although TLR-2 and H\textsubscript{2}R expression was similar for healthy volunteers and IBD patients, cytokine responses were significantly different. Histamine altered TLR-2-induced IL-12, TNF-\textsubscript{a}, G-CSF, IFN-\gamma, MCP-1, IL-1\beta, IL-6 and GM-CSF secretion from healthy volunteers, while similar histamine effects on TLR-2-induced cytokine responses in IBD patients was only observed for TNF-\textsubscript{a} and G-CSF. Histamine mediated these effects primarily through the H\textsubscript{2}R as famotidine negated the differential response.

Patients with IBD display dysregulated expression of TLRs and histamine receptors. The anti-inflammatory influence of H\textsubscript{2}R signaling on TLR-2 innate immune response is abrogated in IBD patients, suggesting that deliberate manipulation of H\textsubscript{2}R-signalling may provide beneficial effects to patients with IBD.

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THE ACTIVATION OF THE SEPTUM LATERAL INCREASED THE RELEASE OF BRAIN HISTAMINE

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The lateral septum (LS) processes the affective significance of sensorial information coming from hippocampus and directs its outputs forward to hypothalamic areas important for motivated, goal-directed behavior. The LS is one of the main afferents to the tuberomammillary nucleus of the hypothalamus (TMN), the only source of brain histamine. Both structures may be implicated in mood and motivation. Brain histamine is important in arousal and alertness. Novelty has motivational effects and can direct and reinforce behavior.

We have proposed that LS could change the activity of histaminergic neurons, modifying histamine release through GABAergic inputs. The reduction in GABAergic input to the TMN should increase vigilance. We demonstrated using immunocytochemistry and electron microscopy that LS input to the TMN region is indeed GABAergic, and these terminals make symmetric synaptic contacts with local dendrites. We measured extracellular histamine and GABA levels in the LS using microdialysis and simultaneously made EEG recording. The reverse microdialysis into the LS with 0.1mM picrotoxin and 10 mM glutamate increased histamine and GABA release in LS as well as increased wakefulness measured with EEG and frequency theta wave.

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Poster presentations
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ROLE OF HISTAMINE H4 RECEPTOR IN NOCICEPTION

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF 1,3,5-TRIAZINE DERIVATIVES – POTENTIAL HISTAMINE H4 RECEPTOR LIGANDS

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P5  A. Krawiec, K. Walas, J. Jochem
INVOLVEMENT OF THE CENTRAL HISTAMINERGIC SYSTEM IN NEUROPEPTIDE Y RECEPTOR-MEDIATED CARDIOVASCULAR EFFECTS IN HAEMORRHAGIC SHOCK IN RATS

INFLUENCE OF CYCLOOXYGENASE INHIBITORS ON ANTIHYPERALGESIC ACTION OF JNJ7777120 IN ADJUVANT INDUCED ARTHRITIS

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ADMINISTRATION OF HISTAMINE TO THE CAT PRIMARY SOMATOSENSORY CORTEX DEPRESSES C-FIBRE EVOKED NEURONAL ACTIVITY VIA H2 RECEPTORS

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**Poster Sessions III and IV**

**Poster Session III: Histamine: Molecular Signaling**

*Chaired by Madeleine Ennis and Arianna Rosa*

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P. Zhao 1, YF. Shao 1, M. Zhang 1, K. Fan 1, XP. Kong 1, R. Wang 1, JS. Lin 2, YP. Hou 1

In spite of the initial and pivotal findings that the newly identified neuropeptide S (NPS) promotes arousal associated with locomotor and anxiolytic-like effects, the mechanisms through which NPS acts to modulate sleep-waking states remain unclear. The present study was undertaken to investigate in the rat the effects of intracerebroventricular injection of NPS on the EEG, sleep-wake cycle, and brain c-Fos expression. NPS at 0.1 and 1 nmol increased significantly wakefulness during the first 2 h postinjection (55 ± 3 and 65 ± 2 min, respectively, vs. 41 ± 3 min seen with saline injections, P<0.01 and P<0.001), accompanied by an increase in EEG high frequency activities (15-60 Hz). In the meanwhile, slow wave sleep and paradoxical sleep decreased significantly. *Ex vivo* double immunohistochemistry of c-Fos coupled with that of histidine decarboxylase (the synthetizing enzyme of histamine) and orexin A in the posterior hypothalamus revealed that, as compared with saline-treated rats, NPS enhanced the number of c-Fos expressing cell bodies of histaminergic neurons by 76% in the ventral part of the tuberomammillary nucleus and 58% in its dorsal part. NPS also enhanced c-fos labelling in orexinergic neurons in the perifornical (+28%), dorsomedial hypothalamic (+24%) and lateral hypothalamic (+14%) areas of the posterior hypothalamus. The NPS-induced c-Fos expression in histaminergic and orexinergic neurons where NPS receptor mRNA is highly expressed, suggests that NPS activates histaminergic and orexinergic neurons to promote wakefulness.

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It was demonstrated that histamine H$_4$ receptor (H$_4$R) antagonists or H$_4$R inverse agonists increase nociceptive thresholds in different kinds of animal pain models. Because the mechanism of this action is still poorly understood, the aim of the present study was to investigate the influence of COX, LOX, NO synthase inhibitors as well as opioid, NMDA, and adrenergic receptor antagonists on the analgesic activity of a H$_4$R inverse agonist, JNJ7777120 (J77).

The studies were performed on male WAG rats. The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal test described by Randall-Selitto and thermal stimuli – tail-flick test. Indomethacin, which is a preferential inhibitor of cyclooxygenase-1 COX-1, but not celecoxib, a relatively selective inhibitor of COX-2, significantly increased analgesic activity of J77. L-NOArg, a non-selective NO synthases inhibitor, potentiated J77 analgesia in both tested methods. It is important to emphasize that MK-801, which is an antagonist of NMDA receptors and yohimbine, α2 receptors antagonist, abolished but an α1 antagonist, prazosin, intensified J77 antinociception on mechanical and thermal stimuli. A non-selective opioid receptor antagonist, naloxone, abolished or increased J77 antinociception in Randall-Selitto or tail-flick models, respectively.

It seems, that H$_4$Rs are engaged in a mechanism of acute pain and this effect may be regulated by the above mentioned pathways.

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Expression of the most recently discovered histamine H₄ receptor (2000/2001) occurs mainly in monocytes, mast cells, eosinophils and basophils [1], suggests that the H₄R is involved in inflammatory processes and immune responses [2]. Recent studies results indicate also that H₄R antagonists have been shown to be effective in several models of pain [3]. As the physiological role of the H₄R is not clear - new, potent and selective ligands are required to investigate its action. Among H₄R ligands already described in the literature a large group of triazine derivatives can be found [4,5,6]. This kind of derivatives has been synthesized in our Department for the past several years.

Presented work involves evaluation of analgesic activity of 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives (TR7 and TR18; 25 mg/kg, i.p.) in rats using mechanical (Randall-Selitto) and thermal (Plantar Test) models of carrageenan-induced hypernociception. Moreover, their anti-inflammatory activity in Zymosan A-induced peritonitis in mice was evaluated. In addition, in vitro preliminary screening of antiproliferative effects of TR7 and TR18 on HEK-293 and IMR-32 cell lines, was also carried out. Compounds examined in the presented studies were selected from the library of compounds synthesized in our Department after preliminary tests conducted in vivo in mice.

The obtained results showed that pre-treatment with TR7 as well as TR18 decreased the intensity of mechanical inflammatory hyperalgesia. In the thermal model, only TR7 increased paw withdrawal latency. Both compounds inhibited cell migration and plasma exudation in the mouse model of peritonitis. In vitro studies of TR7 and TR18 showed a weak antiproliferative effect against both HEK-293 and IMR-32. It can be concluded, that 1,3,5-triazine derivative histamine H₄R antagonists reduce hyperalgesia in in vivo models of inflammation in rats and possess anti-inflammatory activity, which was observed in the mouse model of peritonitis.


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SUBSTANCE P AMPLIFIES CAPSAICIN SIGNALLING IN MOUSE HISTAMINERGIC NEURONS

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The transient receptor potential vanilloid 1 (TRPV1) channel, also called “capsaicin receptor” is activated by noxious heat, protons and capsaicin integrating multimodal peripheral signals to excitation of primary afferent nociceptors in dorsal root, trigeminal and nodose ganglia. For nociception capsaicin-receptor signalling is amplified by local release of histamine (HA) and substance P (SP) [1] with histamine 1 receptors (H1R) and neurokinin 1 receptor (NK1R) involved. TRPV1 is expressed in the caudal hypothalamus [2]. Do central HA neurons, which can synthesize SP[3], participate in capsaicin-receptor signalling in the tuberomamillary nucleus (TMN)? With single-cell RT-PCR we detect TRPV1R in 39%, NK1R in 67% and substance P-mRNA in 61% of HA neurons identified by the expression of histidine decarboxylase (HDC). Most HA neurons are excited by capsaicin 0.1-1µM, the effect is absent in TRPV1 knockout mice. Long-lasting enhancement of firing rate (LLEfr) by capsaicin, observed upto 70 min after capsaicin withdrawal is blocked by the NK1R antagonist CP96345. LLEfr is also seen with NK1R agonist GR73632 (10nM), the effect is abolished by CP96345 (1µM). TRPV1 expression and responsiveness to capsaicin of HA neurons declines with brain maturation whereas substance P signalling remains unchanged. In adult TRPV1−/− mice expression of NK1R is decreased, whereas H1R expression is increased in TMN. In histamine deficient mice (HDC−/−) TRPV1 expression is up-regulated. Thus hypothalamic TRPV1, recruiting SP, influences activity and may affect maturation and connectivity of the histaminergic system.


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INVOLVEMENT OF THE CENTRAL HISTAMINERGIC SYSTEM IN NEUROPEPTIDE Y RECEPTOR-MEDIATED CARDIOVASCULAR EFFECTS IN HAEMORRHAGIC SHOCK IN RATS

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The activation of the central histaminergic system leads to the reversal of haemorrhagic shock in rats and, on the other hand, as we demonstrated recently, neuropeptide Y (NPY) administered intracerebroventricularly (icv) induces a depressor effect in haemorrhagic hypotension. Since histaminergic neurones of the tuberomammillary nucleus receive input from neurones producing NPY localized in the caudal magnocellular nucleus of the hypothalamus, the aim of the present study was to examine (1) cardiovascular effects of NPY receptor antagonists in haemorrhagic shock and (2) a possible involvement of the histaminergic system in their action. Experiments were performed in ketamine/xylazine-anaesthetised male Wistar rats subjected to irreversible haemorrhagic hypotension, with mean arterial pressure (MAP) stabilized at 20-25 mmHg. NPY receptor type 1 and 2 antagonists – BIBP 3226 (25 nmol, icv) and BIIE 0246 (1 µmol, icv), respectively, administered at 5 min of critical hypotension evoked rises in MAP and renal blood flow, with no influence on heart rate. Haemodynamic effects of NPY receptor antagonists were partially inhibited by a pre-treatment with histamine H₁ and H₀/₁ receptor antagonists chlorpheniramine (50 nmol, icv) and thioperamide (50 nmol, icv), respectively, whereas H₂ receptor blocker ranitidine (50 nmol, icv) had no effect. In the control, previously described groups, histamine receptor antagonists given alone did not influence cardiovascular regulation in the used model of haemorrhagic shock. In conclusion, we demonstrate for the first time (1) the pressor effect of centrally acting NPY receptor type 1 and 2 antagonists in haemorrhage-shocked rats and (2) the involvement of the histaminergic system in this action.

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INFLUENCE OF CYCLOOXYGENASE INHIBITORS ON ANTIHYPERALGESIC ACTION OF JNJ7777120 IN ADJUVANT INDUCED ARTHRITIS


Histamine H$_4$ receptor (H$_4$R) antagonists are promising agents that could be used in the treatment of chronic inflammatory diseases because of their anti-inflammatory and antinociceptive activities. The aim of this study was to investigate the possibility of pharmacological regulation of nociceptive pain by histamine H$_4$R antagonists and to evaluate the role of arachidonic acid derivatives in H$_4$R mediated nociception in adjuvant-induced arthritis.

This study was performed on male WAG rats. Animals were immunized with a single subplantar injection of 0.1 ml inactivated Mycobacterium Tuberculosis suspended in incomplete Freund’s adjuvant into the left hind paw. An inflammatory chronic pain was measured by using mechanical stimulation (Randall-Selitto test). An inverse agonist of H$_4$R, JNJ7777120 (J77; 25mg/kg i.p.), a relatively selective inhibitor of COX-1, indomethacin (3mg/kg i.p.), and a selective inhibitor of COX-2, celecoxib (1mg/kg, i.p.), were administered for five consecutive days starting from the 21st day of the experiment.

J77 increased the nociceptive threshold for mechanical stimuli. A premedication with indomethacin, but not with celecoxib, significantly potentiated the antihyperalgesic activity of J77. This observation suggests that COX1 but not COX2 is involved in H$_4$R mediated antihyperalgiesia in the model of inflammatory chronic pain.

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INVLVEMENT OF HISTAMINE H₁ AND H₄ RECEPTORS IN A MURINE MODEL OF ACUTE SKIN INFLAMMATION AND PRURITUS

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The anti-inflammatory effects of histamine H₄ receptor (H4R) antagonists, previously evidenced in various animal models of inflammation, opened new therapeutic options for the treatment of inflammatory/allergic diseases, which are scarcely affected by histamine H₁ receptor (H1R) antagonists. In the present study, both first and second generation H₁R antagonists (pyrilamine, PYR and cetirizine, CET, respectively) and the H₄R antagonist JNJ7777120 (JNJ) were tested either alone or in combination, in the mouse ear edema assay after challenge with local application of 2.5% croton oil (CO). Ear weight and histological tissue damage were investigated at 2h after CO application; the CO-induced scratching was evaluated 1h afterwards. PYR (10 and 30 mg/kg) and JNJ (30 mg/kg), administered subcutaneously (sc), significantly decreased ear edema (45%, 86% and 61%, respectively); when JNJ was dosed in conjunction with PYR 10 and 30 mg/kg, the inhibitions were similar to those observed after PYR alone (58% and 90% vs 45% and 86%). Differently from PYR, CET (10 and 30 mg/kg) scarcely reduced ear edema (20% and 41%, respectively); combined administration of CET plus JNJ did not significantly enhance JNJ-induced effects (69% and 78% vs 61%). These data were confirmed by the histological evaluation of ear tissues. The CO-induced itch was not significantly reduced by JNJ or CET (30 mg/kg sc) alone (30% and 9%, respectively) or by the combined drug administration (35%). As opposed to CET, PYR (30 mg/kg) completely blocked ear pruritus, either alone or plus JNJ. Taken together, these results indicate that both H₁Rs and H₄Rs are involved in the mouse chemically induced skin inflammation; CO-induced pruritus, however, seems to be variably affected by the drug tested. The potential advantage of a combined treatment with H₁R and H₄R antagonists in this skin inflammation model needs further studies.

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H₁ HISTAMINE RECEPTORS IN THE AMYGDALA MEDIATE EMOTIONAL MEMORY DEFICIT IN MICE SUBJECTED TO ELEVATED PLUS-MAZE TESTING

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Some evidence supports the concept that histaminergic neurons influence learning and memory via H₁ histamine receptor activation. The aim of the present study was to verify the effects of microinjected chlorpheniramine (CPA), a histamine H₁ receptor antagonist, on emotional memory of mice re-exposed to the Elevated Plus Maze (EPM). Tests were performed on two consecutive days: Trial 1 (T1) and Trial 2 (T2). Drugs were administered prior to T1 and T2. Twenty-four hours later (i.e., T2) the mice were injected again under the same experimental conditions. Before each trial, mice were microinjected with CPA (0.16 nmol/0.1μl), or saline (SAL) into the amygdala and submitted to the EPM. Anxiety was assessed in the EPM by recording the conventional measures (percentage of open arm entries - % OAE and percentage of time spent in open arms - % OAT) in T1. The decreased open-arm activity (% OAE and % OAT) in T2 was defined as learning and memory index. The data were analyzed using two-way Analysis of Variance (ANOVA) and Duncan’s tests. Post hoc test did not show significant differences between SAL–SAL and CPA–SAL or CPA-CPA groups in T1 for % OAE and % OAT (p>0.05), indicating that the drug did not induce changes in anxiety level. Emotional memory, as revealed by a reduction in open arm exploration between both trials, was present in the SAL-SAL group as well as in the SAL-CPA group (p<0.05). On the contrary, neither the CPA-CPA group nor the CPA-SAL group (p>0.05) exhibited this decrease in open arm activity between both trials, which reveals that CPA impaired emotional memory. No significant changes were observed in the number of enclosed arm entries (EAE), an EPM index of general exploratory activity. Taken together, these results suggest that the H₁ receptors in the amygdala are not implicated in anxiety-like behaviors, but are involved in emotional memory acquisition and consolidation deficits in mice subjected to EPM testing.

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ABT-239 AND DONEPEZIL REQUIRE THE INTEGRITY OF THE CENTRAL HISTAMINERGIC SYSTEM TO EXERT THEIR BEHAVIOURAL AND NEUROCHEMICAL EFFECTS

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Histamine H3 receptors are auto- and heteroreceptors that restrict release of histamine and other neurotransmitters, including ACh. Several results support the involvement of the cholinergic transmission as an essential component in cognitive functions. Thus, the ability of H3 antagonists/inverse agonists to increase ACh release in the cortex and hippocampus, brain areas involved in mnemonic processing, makes the H3 receptor an attractive target for the treatment of cognitive disorders. We addressed the question if H3 antagonists/inverse agonists and acetylcholinesterase (AChE) inhibitors require an intact histamine neuronal system to exert their procognitive effects in the object recognition test (ORT). Systemic administrations in mice of the H3R antagonist ABT-239 (3mg/kg) or the AChE inhibitor donepezil (1 mg/kg) increased the time spent exploring the new object compared to the familiar one. However, these compounds were ineffective in either genetically (HDC-KO) or pharmacologically [by means of i.c.v injection of 5µg α-fluoromethylhistidine (αFMH) an HDC irreversible inhibitor) histamine-deficient mice. Western blot analysis revealed that ABT-239 or donepezil systemic treatments increased GSK-3β phosphorylation in cortical and hippocampal homogenates from normal but not from histamine-deprived mice. Administration of the PI3K inhibitor LY294002 (40 ng, i.c.v) that blocks GSK-3β phosphorylation, prevented the procognitive effects of either drugs in normal mice. ABT-239 also increased HA and ACh release from the prefrontal cortex of freely moving rats, as measured by double probe microdialysis. Conversely, αFMH-treated rats showed ACh and HA release lower than detectable limits. These data suggest that PI3/AKT/GSK-3β intracellular pathway activation is a requisite for the procognitive effects elicited by ABT239 and donepezil and the lack of GSK-3β phosphorylation could be responsible for the lack of efficacy of both drugs on histamine-deficient animals.

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ROLE OF THE CENTRAL HISTAMINERGIC SYSTEM IN THE ANOREXANT EFFECT OF OLEOYLETHANOLAMIDE: A NEUROANATOMICAL STUDY

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Key factors driving eating behavior are appetite and satiety that are controlled by central neurotransmitter systems and peripheral stimuli. The brain histamine (HA) system elicits a hypophagic effect activating H\(_1\)R in restricted hypothalamic regions. Oleoylethanolamide (OEA) is released by enterocytes in response to dietary fat intake and indirectly signals satiety to hypothalamic nuclei. Systemic administration of OEA induces neurons to produce oxytocin in the periventricular nucleus (PVN) and supraoptic nucleus (SON) that in turn mediates the anorexant effect of OEA\(^1\). Our earlier data showed that OEA needs the integrity of the central HA system to induce hypophagia and to activate oxytocic neurons in the PVN. In this study we used an immunohistochemical approach to further understand how the central HA system participates in OEA-induced hypophagia by analysing c-fos expression in several brain regions and the density of oxytocin in the posterior pituitary gland of male histidinedecarboxylase (HDC)-KO mice and WT littermates. Mice were treated i.p. with 10mg/kg OEA or vehicle after 12h food deprivation. After 2h, mice were sacrificed and perfused with 4% paraformaldehyde. Brains and pituitary glands were cryoprotected and cryodissected (40 and 18 micron, respectively). Our data show that OEA increased c-fos expression significantly in the SON (P<0.05), ARC (P<0.05) and BLA (P<0.05) of WT vs saline-treated mice, but not of HDC-KO mice. No differences were observed in the DMH and VMH of either genotype treated with OEA or saline. Analysis of posterior pituitary glands of mice treated with OEA showed a higher density of oxytocin in WT compared to saline treated mice, that is not observed in HDC-KO mice (P<0.05). Our results confirm that the histaminergic system is involved in the anorexiant effects of OEA and that dysregulation of PVN neuronal activity may be responsible for the partial lack of efficacy of OEA in HA deficient mice.

* equal contribution

\(^1\)Gaetani et al. (2010). J Neurosci

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HISTAMINE H₃ RECEPTOR ACTIVATION PREVENTS ADENOSINE A₂A RECEPTOR- MEDIATED FACILITATION OF K⁺-EVDOKED [³H]-GABA RELEASE FROM RAT GLOBUS PALLIDUS SYNPATOSOMES


The globus pallidus (GP) forms part of the basal ganglia, a group of sub-cortical neuronal nuclei involved in the control of motor behavior. The GP possesses a high density of H₃Rs, mostly located on the axons of neurons projecting to the nucleus, as indicated by the very low levels of the corresponding mRNA. The main synaptic afferents to the GP are striato-pallidal GABAergic axons, endowed with adenosine A₂A receptors (A₂ARs). By using rat GP isolated nerve terminals (synaptosomes) in this work we have studied whether H₃R activation affects the previously reported enhancing action of A₂AR stimulation on depolarization-evoked [³H]-GABA release. The A₂AR agonist CGS-21680 (3 and 10 nM) enhanced [³H]-GABA release induced by depolarization with high K⁺ (20 mM) and the effect of 3 nM CGS-21680 was prevented by the A₂AR antagonist ZM-241385 (100 nM). The presence of pre-synaptic H₃Rs on GP nerve terminals was confirmed by the specific binding of N-α-[methyl-³H]-histamine to synaptosomal membranes (maximum binding, Bmax, 1.327 ± 79 fmol/mg protein; dissociation constant, Kd, 0.74 nM, pK₉ 9.13 ± 0.05), which was inhibited by the selective H₃R ligands immepip and clobenpropit (inhibition constants, Ki, 0.28 nM and 8.53 nM, respectively). Perfusion of synaptosomes with the H₃R agonist immepip (100 nM) had no effect on K⁺-evoked [³H]-GABA release, but prevented the stimulatory action of A₂AR activation. In turn, the immepip effect was blocked by the H₃R antagonist clobenpropit (3 μM), which had no significant effect of its own on K⁺-induced [³H]-GABA release. These data indicate that H₃R activation selectively counteracts the facilitatory action of A₂AR stimulation on GABA release from striato-pallidal projections. The GP is critical in the control of the basal ganglia motor output, and the modulation by pre-synaptic H₃Rs of the pallidal GABAergic transmission could therefore contribute to regulate the activity of GP neurons and thus basal ganglia function.

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INTRA-AMYGDALA HISTAMINE IMPAIRS AVERSIVE MEMORY OF MICE IN INHIBITORY AVOIDANCE TASK WHILE NOT IN THE ELEVATED PLUS MAZE

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There is evidence that histamine (HA) modulates learning and memory in different types of behavioral tasks, but the exact nature of this modulation and its mechanisms are controversial. It is known that the amygdala plays a crucial role in emotions and memory processing. Our aim was to investigate whether exogenous histamine (HA) infused into the amygdala modulates differently anxiety-like behaviors and emotional memory of male Swiss albino mice in two distinct tasks, Elevated Plus Maze (EPM) and Inhibitory Avoidance Task (IA). Over two consecutive days, animals received bilateral microinjections of either vehicle – saline or HA (0.1; 0.5 and 1.0 μg by 0.1 μl/side volume) into the amygdala before the behavioral tests were performed. Mice were studied under two paradigms: exposure/re-exposure procedure in EPM, or IA with habituation, electric foot-shock trials 1 and 2 and retention test (without foot-shock). Intra-amygdala microinjection pre-tests of three doses of HA do not interfere in anxiety-like responses and neither do they in mnemonic processing examined with the EPM. Further, the step-through retention latencies increased in all groups compared with its respective trials, except on the mice microinjected with 0.5 or 1.0 μg HA only before the retention test. Thus, HA caused significant amnesia during the session repeated 24 h after training without drugs. This latency was recovered when the drug was administered both pre-trial and pre-test in animals subjected to IA. These findings indicate that the amygdaloid histaminergic system modulates the emotional memory available through inhibitory avoidance, but not in the EPM, besides displaying a state-dependence mechanism for evocation stage. Histamine impaired retrieval process in the IA and we suggest a distinct histaminergic influence for different emotional pathways.

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EFFECT OF HISTAMINE H3 RECEPTOR ACTIVATION ON RAT NUCLEUS ACCUMBENS DOPAMINERGIC TRANSMISSION


The nucleus accumbens (NAcc), involved in motivated behaviors, expresses high levels of histamine H3 receptors (H3Rs) and recent evidence supports the location of these receptors on NAcc dopaminergic terminals. In this work, we therefore set out to determine the effect of H3R activation on rat NAcc dopaminergic transmission by analyzing [3H]-dopamine uptake by synaptosomes and dopamine synthesis and depolarization-evoked [3H]-dopamine release in slices. The presence of the H3Rs was confirmed by the specific binding of N-\alpha-[methyl-3H]histamine to membranes of the NAcc (maximum binding, B_{max}, 191 ± 22 fmol/mg protein and dissociation constant, K_d, 2.09 nM; pK_d 8.68 ± 0.07), which was inhibited by the H3R ligands RAMH (pKi 8.82 ± 0.17), clobenpropit (pKi 8.07 ± 0.18) and ciproxifan (pKi 8.17 ± 0.12). The inhibition of [3H]-dopamine uptake by NAcc synaptosomes followed the rank order of potency GBR-12909 (IC_{50} 2.4 nM, pIC_{50} 8.62 ± 0.15) >> fluoxetine (IC_{50} 3,890 nM, pIC_{50} 5.41 ± 0.11) ≈ desipramine (IC_{50} 5,248 nM, pIC_{50} 5.03 ± 0.30), indicating that [3H]-dopamine is selectively taken up by dopaminergic nerve terminals. Specific [3H]-dopamine uptake by NAcc synaptosomes was not affected by the selective H3R agonist RAMH (10^{-10}-10^{-6} M). In slices labeled with [3H]-dopamine perfusion with RAMH (1 μM) had no significant effect (91.4 ± 4.5% of controls) on [3H]-dopamine release evoked by depolarization with high K⁺ (30 mM). The blockade of dopamine D2 autoreceptors with sulpiride (1 μM) enhanced K⁺-evoked [3H]-dopamine release, but under these conditions RAMH also failed to affect [3H]-dopamine release. Dopamine synthesis is evaluated by determining L-DOPA accumulation in slices incubated with the DOPA decarboxylase inhibitor NSD-1015, and preliminary data show that neurotransmitter synthesis is reduced by H3R activation. These data indicate that in rat NAcc H3Rs do not modulate dopamine uptake or release, but regulate neurotransmitter synthesis.

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THE ROLE OF CEREBELLAR HISTAMINERGIC SYSTEM ON EMOTIONAL MEMORY CONSOLIDATION

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This study investigated the function of cerebellar histaminergic system on emotional memory consolidation. The cerebellar vermis of male mice were implanted with guide cannulae, and after three days of recovery, the animals were submitted to the elevated plus maze (EPM) or the inhibitory avoidance test (IA). Immediately after the first day, animals received microinjection of histaminergic drugs: experiment 1, animals received microinjections of saline (SAL) or histamine (HA) (0.54, 1.36, 2.72, and 4.07 nmol/0.1µl); experiment 2, animals received microinjections of SAL or the H₁ antagonist chlorpheniramine (CPA); experiment 3, SAL or the H₂ antagonist ranitidine (RA); experiment 4, SAL or HA 5 minutes after a pretreatment with CPA or SAL; and experiment 5, SAL or HA 5 minutes after a pretreatment with RA or SAL. The results of experiment 1, in the EPM, showed that mice microinjected with HA 2.72 and 4.07 nmol did not decrease open arm exploration on trial 2; which indicates that histamine induced a dose-dependent inhibitory effect on memory consolidation. In the IA, results showed that 1.36 nmol histamine facilitated memory consolidation in this task. In the experiment 2, microinjections with CPA did not present behavioral effects in the EPM or in the IA at the doses used. The results of experiment 3 showed that 5.7 nmol RA impaired memory consolidation on both protocols. The experiment 4 demonstrated that animals treated with HA did not reduce the avoidance to the open arms on retesting, and indicated that the pretreatment with CPA reverted histamine-induced impairment on memory consolidation, which suggests that histamine effect on the EPM was mediated by H₁ receptors. Whereas the experiment 5 showed that the effect of histamine on the IA was mediated by H₂ receptors, since the pretreatment with RA did prevent the enhancement in memory consolidation. Our results suggest a different role of the cerebellar histaminergic system in tasks involving anxiety or fear.

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CHARACTERISATION OF THE PROMOTER REGION OF THE HUMAN HISTAMINE 4 RECEPTOR

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The human histamine 4 receptor (HRH4) is the newest member of the histamine receptor family and is mainly expressed in haematopoietic cells. The role of this receptor is still under investigation and so far it has been related to pathological conditions such as inflammation, allergy, autoimmune diseases and cancer. The promoter region of this gene is characterised by a complex length variation and single nucleotide polymorphisms (SNP). This study aims to identify the length of the active promoter region of this gene and to identify the significance of a microsatellite length variation. The promoter region of the HRH4 has been analysed in silico in previous published studies, however the active promoter was never verified experimentally. Polymerase chain reaction and capillary electrophoresis were used to genotype HapMap samples in order to characterise the promoter region. A potential transcription region was identified by using the bioinformatics database Promoter 2.0 and fragments of the promoter region were cloned into a reporter vector, pGL3-basic (Promega) with or without exon 1. Various promoter lengths where analysed with the Dual-Glo luciferase assay using HEK293T cells and evaluated using one-way ANOVA, followed by a Dunnett multiple comparison test. In addition, a microsatellite, variable-number of triplet repeat (VNTR) expansion ranging from 10 to 19 repeats was identified as well as a further AC/CA polymorphism in this highly polymorphic region. Functional implication of the polymorphisms were evaluated. The functional promoter was identified as -705 bp to +194 bp with a $P < 0.001$ when compared to the promoter-less pGL3-basic vector.

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CHARACTERIZATION OF H\(_2\) HISTAMINE-RECEPTOR OVEREXPRESSING MICE

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In mouse cardiac preparations, histamine is ineffective, presumably because of lack of receptor protein expression. Hence, we have generated transgenic mice (TG) with cardiac specific overexpression of the human H\(_2\) receptor. In TG (but not WT) hearts, positive immunostaining for the overexpressed receptor was observed in both the myocardium and atria. In all WT and TG probes (myocardium and atria), positive immunostaining for histidine decarboxylase and histamine was noted. Moreover, HPLC-MS analysis revealed measurable amounts of histamine in isolated cardiac myocytes. To further characterize the functionality of the overexpressed receptor, we have compared the effects of histamine and the H\(_2\) receptor agonist dimaprit. In isolated electrically stimulated left atrial preparations, dimaprit exerted a concentration dependent positive inotropic effect (PIE) with a \(-\log EC_{50}\) of 6.4±0.2 (for comparison: 6.7±0.2 for histamine) that was inhibited by the H\(_2\) antagonist cimetidine (p<0.05; n=9). In isolated spontaneously beating right atria, dimaprit elicited a cimetidine-sensitive (p<0.05) positive chronotropic effect (PCE) with an \(-\log EC_{50}\) of 6.9±0.5 (n=6-7). In right atrial preparations of TG (n=15), more often spontaneous arrhythmias were noticed compared to WT (n=10) (p<0.05). In isolated perfused TG hearts, we noted homologous desensitization of the PIE of histamine by pretreatment with 100 μM histamine for 30 min (p<0.05; n=5). Using echocardiography, dimaprit (100 μl; 10 mM) increased heart rate from 493 ± 8 bpm to 542 ± 15 bpm (p<0.05; n=5) and ejection fraction from 60 ± 2.1 % to 79 ± 3.1 % (p<0.05; n=5) in TG but not in WT mice. These PIE and PCE were blocked by cimetidine (100 μl; 50 mM). The present data indicate that we have successfully developed a new mouse model to study cardiac H\(_2\) receptors with the expected agonist and antagonist sensitivity in vitro and in vivo (supported by DFG).

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**H₄ RECEPTOR IS INVOLVED IN EPITHELIAL TO MENSENCHYMAL TRANSITION-RELATED RESPONSES VIA Src/ERK SIGNALING IN MDA-MB-231 CELLS**

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In previous studies we demonstrated in MDA-MB-231 breast cancer cells that 1 µM histamine (HA) and H₄ agonists stimulate some cellular events concerning the epithelial-mensenchymal transition (EMT), a program activated during tumor progression. We also determined that 1µM HA stimulates Src phosphorylation, crucial for MDA-MB-231 cells migration. The aim of this work was to evaluate whether H₄ receptor activation is involved in EMT related responses. We examined the expression and subcellular localization of β-catenin, and the phosphorylation/activation of Src and ERK1/2.

Cells were serum-starved and then treated with 1µM HA or 10 µM H₄ agonists (Clobenpropit or VUF 8430, p<0.05 vs control) plus 10% FBS for 15 min. Src phosphorylation evaluated by Western blot showed a similar increase in P-Src levels in cells treated with HA or H₄ agonists. Cells treated with H₄ agonists showed Src labeling on cell membrane by indirect immunofluorescence (IIF) confirming the Src activation. There was also an increase in the number of migrated cells in the presence of HA or the H₄ agonists, determined by transwells units (p< 0.05). This increase was blocked by the H₄ antagonist JNJ7777120 (10µM). The Src inhibitor PP2 reduced the enhancement of P-Src expression and also cell migration (p<0.05 vs control).

P-Src phosphorylates substrates such as β-catenin which translocates to nucleus and modulates EMT related genes expression. Nuclear and perinuclear β-catenin localization was observed by IIF in cells treated for 24 h with HA or H₄ agonists. The combined treatment with PP2 or JNJ7777120 reversed these effects.

Src phosphorylation induced by H₄ agonists was not modified by the MEK inhibitor PD98059. However, ERK1/2 phosphorylation induced by H₄ agonists was decreased in the presence of PP2 after 15 min (p<0.05). In addition, PD98059 hindered the enhancement of cell migration induced by H₄ agonists (p<0.01).

Our results support the involvement of the H₄ receptor in MDA-MB-231 cells migration via Src/ERK signaling.

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NEW FINDINGS ON THE CELLULAR EFFECTS OF NEWLY SYNTHETIZED HISTAMINE IN MAMMALIAN CELLS

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Histamine (HIS) is the most multifunctional biogenic amine. It is synthetized by histidine decarboxylase (HDC) in a reduced set of mammalian cell types. Mast cells and histaminergic neurons store HIS in specialized organelles until the amine is extruded by exocytosis; however, other immune and cancer cells are able to produce but not store HIS. The intracellular effects of HIS are still not well characterized, in spite of the physiopathological relevance of the amine. Multiple functional relationships exist among HIS metabolism/signaling elements and those of other biogenic amines, including growth-related polyamines. In a previous work, we obtained the first insights for an inhibitory effect of newly synthetized HIS on the cell cycle of non-fully differentiated mammalian cells. In the present work, we describe progress on this line. HEK293 cells were transfected to express active and inactive versions of GFP-human HDC. Cells expressing GFP fusion proteins were sorted by flow cytometry and their relative levels of protein expression related to cell signaling were measured using an Antibody Microarray. Experimental results were analyzed in terms of protein-protein and functional interaction networks. Key facts were experimentally validated by different approaches. The analyses uncover cross-talk mechanisms among biogenic amine-related pathways and provide new clues on the molecular mechanisms underlying the regulatory effects of intracellular newly synthetized HIS on cell proliferation/survival, cell trafficking and protein turnover. This information is especially interesting for emergent and orphan immune and neuroinflammatory diseases.

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IN VITRO AND IN VIVO CHARACTERIZATION OF ST-1006: A POTENT HISTAMINE H₄ RECEPTOR AGONIST IN THE MOUSE

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Pharmacological assays performed in transfected cell lines characterized ST-1006 (N⁴-(2,6-dichlorobenzyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine) as selective partial histamine H₄ receptor (H₄R) agonist at the human H₄R (Kᵢ = 12 nM; intrinsic activity = 0.28) and at the mouse H₄R (Kᵢ = 8 nM; low intrinsic activity). Recently discrepancies between the efficacies of H₄R agonists obtained from assays in transfected cell lines and assays in native cells have been shown in the human system. In the mouse ST-1006 has not been characterized yet. Since the H₄R displays substantial pharmacological species variation, it is important to characterize H₄R ligands in each species of interest.

As functional readout we analyzed the chemotactic properties of ST-1006 (1 and 10 µmol/l) in NIH-3T3-cells transfected with the recombinant murine H₄R, freshly isolated murine natural killer cells (NK cells) and bone marrow derived macrophages. Expression of the H₄R has been confirmed by RT-PCR. ST-1006 (1 and 10 µmol/l) induced significantly increased migration of H₄R-transfected NIH-3T3-cells, NK cells and macrophages. This effect was not seen in NIH-3T3-cells transfected with murine H₁R and in cells pre-incubated with the H₄R antagonist JNJ7777120 (10 µmol/l).

To test the in vivo activity of ST-1006 in the mouse, itch-inducing properties were analyzed after intradermal injection of ST-1006 (5 and 50 nmol/l) into the back skin of female BALB/c mice. ST-1006 induced a robust and dose-dependent scratching behavior. This study identifies ST-1006 as a suitable pharmacological tool to study the function of the H₄R in murine cells and mouse models of allergic-inflammatory and pruritic diseases.

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EFFECTS OF LONG-TERM (R)-α-METHYLHISTAMINE ADMINISTRATION ON MUCUS-SECRETING AND PROLIFERATIVE CELLS IN THE RAT GASTRIC MUCOSA

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(R)-α-methylhistamine, selective agonist of histamine H₃ receptors, potently stimulates cell proliferation and migration in the rat gastric fundus, as early as 1 h after a single intragastric administration. The aim of the present study was to determine the possible influence of long-term administration of (R)-α-methylhistamine on mucus-secreting and proliferative cells in the rat fundic mucosa. (R)-α-methylhistamine was administered daily by intragastric route to male Wistar rats for 1 and 4 weeks at the doses of 10 and 100 mg/kg. All the animals received 5-bromo 2’ deoxyuridine, (BrdU), 200 mg/kg i.p. 2 h before sacrifice. Gastric tissue was processed for histology and immunohistochemistry. In control rats the number of BrdU-positive cells was 1.04 ± 0.18 per gland. Following 1 week administration of (R)-α-methylhistamine, 10 and 100 mg/kg, the number of BrdU-positive cells was significantly increased respectively by 88% and by 116%, compared with saline-treated controls. Following 4 weeks of daily (R)-α-methylhistamine administration, there was a 31% decrease of BrdU-positive cells in rats receiving the dose of 10 mg/kg and a moderate increase by 48% in rats receiving the dose of 100 mg/kg, compared with the corresponding controls. Administration of (R)-α-methylhistamine for 4 weeks was associated with the down-regulation of MUC5 expression. MUC5-expressing cells were significantly reduced by 62% and by 51% following daily administration of 10 and 100 mg/kg (R)-α-methylhistamine respectively, compared with saline-treated controls. MUC5 was expressed throughout the pit region up to surface mucous cells, while in (R)-α-methylhistamine-treated rats, it was expressed only in the low and mid portion of the pit region. The data support a role for (R)-α-methylhistamine in regulating cell proliferation and in modifying the distribution pattern of mucus-secreting cells in the gastric mucosa.

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Human lung mast cells (HLMC) play a significant role in asthma being a source of pro-inflammatory mediators such as histamine, cysteinyl-leukotrienes (CysLT) and a particularly rich source of the prostaglandin, PGD$_2$. PGD$_2$ is derived from arachidonic acid by the action of cyclooxygenase (COX), of which two isoforms, COX-1 and COX-2, have been identified. The principal aim of this study was to determine the isoform of COX responsible for PGD$_2$ generation in HLMC.

HLMC were generated by physical and enzymatic disruption of human lung tissue. HLMC were further purified by Percoll density gradient centrifugation followed by immunomagnetic separation. HLMC were incubated (15 min) with or without the COX inhibitors (0.01-1µM) indomethacin (non-selective), FR122047 (COX-1 selective) or celecoxib (COX-2 selective) before challenge with anti-IgE (2µg/ml; 25 min). Wortmannin (1µM) was used as a positive inhibitory control. Histamine released into supernatants was assayed using an automated fluorometric technique. PGD$_2$ and CysLT generation were determined using commercially available ELISAs. COX expression was determined by RT-PCR and immunoblotting. To determine statistical significance, ANOVA was performed (GraphPad V6).

Indomethacin (non-selective) and FR122047 (COX-1 selective) significantly (P<0.05) inhibited PGD$_2$ generation, whereas celecoxib (COX-2 selective) was ineffective (n=4). By contrast, none of the COX inhibitors had an effect on IgE-mediated histamine release (n=5). None of these compounds had an inhibitory effect on IgE-mediated CysLT release (n=4) although, paradoxically, celecoxib (1µM) caused a significant (P<0.05) enhancement of IgE-mediated CysLT generation. At the mRNA level (n=3) both isoforms of COX were strongly expressed. At the protein level (n=4) COX-1 was strongly expressed whereas COX-2 was variably expressed.

These data indicate that although HLMC may express both isoforms of COX, the synthesis of PGD$_2$ is predominately mediated by COX-1.

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EOSINOPHILS ADHESION TO ENDOTHELIUM CELLS: INNOVATIVE MODEL IN ESTIMATING ROLE OF HISTAMINE IN PROCESS OF INFLAMMATION.

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Histamine is well known biogenic amine, playing an important immunomodulatory role in inflammation. Recently it has been clearly shown that histamine H4 receptor is mainly responsible for histamine effects on immune cells (Zampeli et al., 2009). Regardless of published data, still there are insufficient detailed analyses regarding the role of histamine receptors in leukocyte functions, mainly due to the difficulty of obtaining pure and functional populations of these cells (Seifert et al., 2012). The aim of the presented study was to estimate the role of histamine and histamine H4 receptor in the process of eosinophils adhesion to endothelium cells. Human eosinophils were isolated from fresh human blood by ficoll-Paque density gradient separation, followed by negative immunomagnetic cell sorting. Viability of the isolated cells have been determined using trypan blue exclusion test and MTS assay. Functional properties of isolated eosinophils have been assessed by calcium flux assay and EPO release experiments. Eosinophils adhesion to endothelium cells was evaluated by isolated eosinophils co-culture with the EA.hy.296 endothelium cell line. Trypan blue staining and MTS assay revealed high viability of isolated cells, lasting for approximately 2.5 h after isolation. Functional test proved the ability of eosinophils to degranulate EPO. fMLP had concentration dependent effect on eosinophils adhesion to endothelium cells. Stimulation with histamine and selective antagonist of human H4 histamine receptor – JNJ7777120 resulted in variable eosinophil adhesion efficiency. These observations suggest that histamine effect on eosinophils adhesion have very subtle characteristics. We hope that the newly developing model of eosinophils adhesion to endothelium cells will help in understanding the physiological role of histamine, as well as that it will provide a new tool in pharmacological study of different receptor agonists and antagonists.

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HISTAMINE RECEPTORS IN A SIALOADENITIS INFECTION MODEL BY STAPHYLOCOCCUS AUREUS.

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Salivary glands are exocrine glands, which produce and secrete saliva into the mouth. Bacterial infections of the submandibular and parotid glands are the most frequent cause and sites of sialadenitis. The most common pathogens associated with acute gland infection are Staphylococcus aureus and anaerobic bacteria. The goal of the present work has been to establish an infection model of rat submandibular glands and to study the effect of histamine on proinflammatory markers during the course of infection. We have determined nitric oxide (NO), prostaglandin E2 (PGE2) and metalloproteinase-3 (MMP-3) by ELISA in submandibular glands incubated in the presence or absence of histamine (10^-10^-10^-5M) of male Wistars rats inoculated or not with S. aureus (ATCC 25923), and sacrificed 48 hours post-inoculation (pi). The salivary glands infection model developed in our lab produces a classic non-specific acute inflammation, from 24 hours after inoculation of S. aureus, with a peak between 48 and 72 hours pi, when abscesses become apparent. Infected glands showed significantly increased levels of NO, PGE2, and MMP-3 while histamine further increased NO, PGE2, and MMP-3 levels in both healthy and infected glands. The increase of proinflammatory markers is mediated by the activation of NOS, PLA2 and COX and increased [Ca2+]i through H1 and H4 receptor activation in healthy and infected glands, respectively. In conclusion, the action of histamine involved binding and activation of H1 receptor in healthy gland and H4 receptor in infected submandibular gland provoking a generation of NO and PGE2 and increased MMP-3 production.

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TOPICAL APPLICATION OF H₄R LIGANDS PREVENTS ALVEOLAR BONE LOSS AND SALIVARY GLAND ALTERATIONS IN A RAT MODEL OF PERIODONTITIS

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Periodontitis is a chronic inflammatory disease of the supporting structures of the teeth initiated by pathogenic bacteria, which often leads to tooth loss. Its significant impact on the patient's general health and quality of life point toward the development of more effective management for this disease. Recently, we have reported that systemic administration of histamine and JNJ7777120 reduced experimental periodontitis (EP)-induced damage on submandibular gland (SMG) and periodontal bone structure.

The aim of this study was to investigate the effects of the topical administration of the H₄R ligands, clozapine and JNJ10191584 (JNJ101), on EP-induced histological, functional and inflammatory alterations in SMG, periodontal bone and gingiva. Bilateral EP was induced for two weeks by placing a cotton thread ligature around the neck of both first lower molars in anesthetized male rats. Clozapine (0.25%) and JNJ101 (2.5%) in the form of gel were administered topically immediately after ligature placement and daily after periodontitis induction for 2 weeks.

Results indicate that both compounds diminished the lingual and vestibular bone loss determined as distances between the cemento-enamel junction and the alveolar crest (0.76 ± 0.03 mm in clozapine group, P<0.001; 0.80 ± 0.04 mm in JNJ101 group, P<0.01 vs. 0.98 ± 0.04 mm in EP group) and reduced the increased PGE₂ and inflammatory infiltration in gingival tissue of rats with EP. In addition, EP produced a 2-fold decrease in methacholine-induced salivation, increased PGE₂ levels and induced vacuolization, apoptosis and areas of necrosis and focal hemorrhage in SMG. On the other hand, H₄R ligands reversed almost completely the salivation reduction produced by EP, preserving the histological characteristics and also reducing the EP-induced PGE₂ levels in SMG.

We conclude that topical administration of H₄R ligands may have the potential to effectively treat periodontal disease by decreasing inflammation of supporting tissues of teeth and preserving alveolar bone and salivary gland tissues.

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THE INVOLVEMENT OF H3 HISTAMINE RECEPTORS IN IGE SYNTHESIS

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In our previous investigations it have been shown that histamine acting via several types of receptors is involved in the regulation of IgE synthesis in both atopic and different non-atopic diseases. Through recent cloning of human histamine H3 and H4 receptors new studies have suggested that dual H3/H4 receptor antagonists may possess a wide range of pharmacological activities including effects on IgE synthesis in atopic and non-atopic diseases. There are controversial data on the distribution of H3 receptors on the different lymphoid and non-lymphoid cells as well as the possible involvement of H3 receptors in the synthesis of IgE antibodies. The goal of this investigation was to evaluate the IgE-modulatory activity of R-alpha-methylhistamine – the histamine agonist selective for the receptor subtype H3. The supernatants after 9-days culture of peripheral blood mononuclear cells (PBMC) from 10 healthy donors as well as 12 allergic subjects sensitive to ragweed pollen obtained during the pollen season were assayed for total IgE by the CAP FEIA method (Phadia). Concentration of pro- and anti-inflammatory cytokines (IL1β, TNFα, IL4 and INFγ) were assessed by ELISA in supernatants of PBMC pre-treated with the H3 agonist R-alpha-methylhistamine (RMH). Loratidine and Cimetidine were used as H1 and H2 histamine receptor antagonists, respectively. In the preliminary investigations, it was shown that histamine dose-dependently modulate an IgE synthesis in healthy donors as well as 12 allergic subjects. At 10^-5 M concentration, histamine reduced IFNγ levels from 8.12±0.1 pg/ml to 6.9±0.12 pg/ml while increasing IL-4 and IL-10 levels (p<0.01). It has been shown that RMH (10^-5 M) decreased IgE synthesis and at a low concentration (10^-8 M) highly increased IgE synthesis up to 4-fold. Pretreatment of PBMC with combinations of H1 and H2 antagonists modulated IgE synthesis in PBMC cultures of healthy donors. The high concentrations of RMH in this cell culture highly increased IgE response, while a low concentration had an opposite effect. Allergen-induced cells of ragweed sensitive patients responded to RMH less than healthy donor’s cells. At the same time, RMH dose-dependently decreased IgE synthesis in allergic patient’s cell culture pretreated with H1 and H2 antagonists. IgE-modulatory effects were highly cytokine dependent, especially on the levels of IL4 and INFγ. Thus, histamine H3 receptors may be involved in the regulation and modulation of IgE synthesis both in healthy donors and allergic patients. The effects of H3 histamine receptor agonist R-alpha-methylhistamine on IgE synthesis by PBMC is dependent on the level of IgE-regulatory cytokines.

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Histamine is an important mediator of biological functions and present in high amounts in inflammatory skin lesions. Such skin lesions evoke a migration of monocyte precursor cells, in part via histamine induced chemotaxis, into the inflamed tissue whereby they differentiate into macrophages. The expression and function of the histamine receptors, especially the histamine H\(_4\) receptor (H\(_4\)R), has already been determined on monocytes and various subtypes of antigen presenting cells in our previous studies. However, the effects of histamine on the two major subtypes of human macrophages (M1 resp. M2 macrophages) have not been studied in detail yet. Therefore, our aim was to assess a functional role of the histamine receptors, with focus on the histamine H\(_4\) receptor, on these professional phagocytes. Here we could show that polarized M1 and M2 macrophages express the H\(_1\)R, the H\(_2\)R and the H\(_4\)R but not the H\(_3\)R on mRNA level. On M2 macrophages we observed an up-regulation of the H\(_1\)R and H\(_4\)R upon activation with IL-4. Interestingly we could show that the phenotype of M1 and M2 macrophages was significantly altered when H\(_4\)R ligands were added continuously to the media during the period of differentiation. A significant up-regulation of the macrophage marker CD68 and a down-regulation of CD163 were detected by flow cytometry in response to treatment with the H\(_4\)R agonist. Furthermore, fully differentiated macrophages were stimulated and the cell free supernatants were analyzed by ELISA. When stimulated with IFN-\(\gamma\) and LPS in the presence of histamine or a H\(_4\)R agonist, M1 macrophages produced substantially lower amounts of the chemokines CCL4 and of interferon-\(\gamma\) induced protein 10 (IP-10, CXCL10). In conclusion, we could show that the H\(_4\)R is functionally expressed on activated macrophages. The down-regulation of CCL4 and IP-10 will lead to decreased migration of immune cells (particularly Th1 lymphocytes) to the site of inflammation and might have implications for the treatment of allergic diseases since H\(_4\)R agonists may attenuate the inflammatory response.
BASOPHIL DEGRANULATION AND ACTIVATION IS REGULATED VIA THE H₄ RECEPTOR

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Basophils, first described by Paul Ehrlich in 1879, circulate at relatively low levels in the peripheral blood and migrate to sites of inflammation. Like mast cells basophils abundantly express the high-affinity IgE-receptor (FcεRI) on their cell surface. Here we investigated the role of the histamine receptors, particularly of the histamine H₄ receptor, in modulating basophil activation and degranulation. We could show that highly purified basophils express the H₁R, the H₂R and the H₃R but not the H₅R on mRNA level by means of real time PCR. As a measure of degranulation sulfidoleukotrienes, produced by isolated IL-3 primed leukocytes, were determined by ELISA. The expression of basophil specific surface markers was evaluated by flow cytometry. Cross-linking of the FcεRI upon stimulation with a specific antibody is a strong activation signal for basophils, resulting in the release of mediators stored in basophil granules such as histamines and proteases and de novo synthesis of mediators such as sulfidoleukotrienes which include leukotriene C₄ (LTC₄) and its derivates LTD₄ and LTE₄.

In this study, we determined that simultaneous treatment with histamine or a specific H₄R agonist significantly reduces the synthesis and release of sulfidoleukotrienes in these highly activated basophils. In patients suffering from bee or wasp venom allergy the allergen-dependent degranulation and sulfidoleukotriene liberation were also decreased by treatment with histamine or the H₄R agonist. Basophils stimulated with histamine or the H₄R agonist alone showed no signs of degranulation or activation. Furthermore, an up-regulation of the activation marker CD203c in response to H₄R stimulation was detected. These data imply that the H₄R regulates IgE-dependent processes and represent an interesting novel mechanism how histamine prevents an overwhelming reaction in a negative feed back loop. These findings will contribute to a better understanding of the complex interplay of inflammatory mediators such as histamine during allergen exposure at the site of allergic inflammation.

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EXPERIMENTAL AUTOIMMUNE MYOCARDITIS IN RATS IS ATTENUATED BY H₄ HISTAMINE RECEPTOR MODULATION

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The histamine H₄ receptor (H₄R) has an important role in inflammation and immune modulation. Here, we have examined its involvement in experimental autoimmune myocarditis (EAM), a model of postinfectious myocarditis and cardiomyopathy progressing to dilated cardiomyopathy in humans. EAM was induced with commercial porcine cardiac myosin in male Lewis rats. Two ligands ST-994 and ST-1012 were used to modulate the H₄R. The drugs (0.6 mg/kg/day for 2 weeks) were applied with ALZET minipumps implanted directly post-immunization. The severity of EAM was monitored by spectrophotometric assay of ceruloplasmin as an acute phase reactant while by ELISA kits of interleukin 1β, interleukin 6 and TNFα plasma levels. The rats were sacrificed 4 weeks post immunization. Macroscopic and histopathological examination of the hearts was performed. Paraffin sections were stained with H&E, Masson trichrome and alcian blue. Other sections were processed for immunohistochemical demonstration of CD3 antigen on reactive T cells, and CD68 antigen on macrophages in tissue with lymphoid infiltrates, H4R (C-20) on leukocytes, and troponin C to elucidate heart muscle damage.

Treatment with the ligands affecting histamine signaling via H₄R attenuated EAM; the inflammation, myocardial damage and fibrosis were reduced and survival rate was increased as compared to untreated counterparts. On the basis of these results, the clinical benefits of H₄R modulation in autoimmune myocarditis could be inferred.

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SALSOILINOL-INDUCED NEUROINFLAMMATION

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Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, SAL) is an endogenous compound thought to be involved in the etiology of Parkinson’s disease (PD). Neuroinflammation is thought to be a major contributor to the neuronal degeneration in PD. The alteration of inflammatory cytokines in the brain, cerebral spinal fluid and plasma of PD patients supports the existence of functional connections between the immune and nervous systems. In animal studies, chronic administration of SAL induced parkinsonian-like symptoms. However, little has been known about the effects of SAL on the cytokine production or hypothalamo-pituitary axis (HPA) activation.

The aim of the study was to evaluate the influence of exogenous SAL on serum levels of corticosterone, CRF and IL-1β.

Wistar rats were subjected to intraperitoneal dosing of SAL (200 mg/kg) with osmotic mini-pumps for 2 (S1, n=8) or 4 weeks (S2, n=8). An equivalent group of rats served as the control (C). At the end of the experiment blood samples were collected and assayed by ELISA.

The serum levels of CRF, corticosterone and IL-1β were elevated in both salsolinol-treated groups in comparison with the C group (S1=0.0944ng/ml ± 0.062, S2=0.0916ng/ml ± 0.036, C=0.0708ng/ml ± 0.025; S1=34.29ng/ml ± 3.7, S2=28.24ng/ml ± 6.2, C=16.94ng/ml ± 9.9; S1=455.68pg/ml ± 134.6, S2=491.62pg/ml ± 120.4, C=321.79pg/ml ± 122.6, respectively). No differences between S1 and S2 groups were observed.

Peripheral SAL administration evokes changes in HPA activity. Our previous results showed that SAL causes mast cells (MC) degranulation in the gut. Once activated, MC may secrete a range of neurosensitizing and proinflammatory molecules, increasing gut-blood and blood-brain barrier permeability. For example, IL-1β is involved in the activation of the HPA, by stimulating hypothalamic CRF secretion and/or by activation of an intra-adrenal CRF/ACTH system. These results serve as an additional support for the existence of a relationship between the nervous, neuroendocrine and immune systems.

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ADMINISTRATION OF HISTAMINE TO THE CAT PRIMARY SOMATOSENSORY CORTEX DEPRESSES C-FIBRE EVOKED NEURONAL ACTIVITY VIA H₂ RECEPTORS

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Histamine in the brain can be released from the nerve terminals originating from histaminergic neurons in the tuberomamillary nucleus of the hypothalamus. Central administration of histamine produces antinociception in formalin or carrageenan-induced hyperalgesia via H₁ and H₂ receptors in rodents. However, central H₁ antagonism produces analgesic effects. Interaction between histamine and morphine, and histamine enhancing stress-induced analgesia (SIA) have been postulated via H₂ receptors. The present study used extracellular recordings of the C-fibre stimulation-evoked single unit discharge in the primary somatosensory area (SI) of the cat cortex to investigate histamine analgesic effects and its receptor mediation.

Selective electrical stimulation of C-fibres in the saphenous nerve induced neuronal discharges recorded in the SI cortex of the anesthetized cat. A normalized cross-covariance function was used to enhance quantitative analysis of single unit discharges and drug actions. Histamine, the H₁ antagonist diphenhydramine or the H₂ antagonist cimetidine were microelectrophoretically applied onto the recorded unit through the multi-barrel microelectrode. Histamine caused significant inhibition of C-fibre-evoked single unit discharges in an ionophoresis current-dependent and reversible manner. Neither H₁ nor H₂ antagonist alone had effects on basal and the C-fibre–evoked discharges, indicating little tonic effects of endogenous histamine in the SI area. The histamine-induced inhibition of neuronal discharges was reversed by co-ionophoresis of cimetidine but not diphenhydramine, indicating selectively via activation of H₂ receptors. Recently we reported that SIA generation requires intact hypocretin/orexin neurons, which directly innervate and stimulate histamine neurons. Therefore, studies on interaction between histamine and hypocretin in the modulation of SIA and broad nociception, particularly have implications in the quest for more effective analgesics.

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PITOLISANT, AN INVERSE AGONIST OF THE HISTAMINE H3 RECEPTOR: AN ALTERNATIVE FOR NARCOLEPSY-CATAPLEXY IN TEENAGERS WITH REFRACTORY SLEEPINESS

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Narcolepsy is a rare disabling sleep disorder characterized by excessive daytime sleepiness and cataplexy (sudden loss of muscle tone). Drugs such as pitolisant, which block histamine H3 autoreceptors, constitute a newly identified class of wake-promoting agents because they increase brain histamine and enhance wakefulness in animal and human adult narcolepsy. We report our experience with the off-label use of pitolisant in 4 teenagers with narcolepsy/cataplexy with severe daytime sleepiness, refractory to available treatments (modafinil, methylphenidate, mazindol, sodium oxybate, and D-amphetamine).

All teenagers developed their disease during childhood (11.3 T 2.4 years; 50% boys) and were 17.3 T 0.8 years old at the time of pitolisant therapy. Pitolisant treatment was increased from 10 to 30 mg (n = 1) and 40 mg (n = 3). The adapted Epworth Sleepiness Score decreased from 14.3 T 1.1 to 9.5 T 2.9 (P = 0.03) with pitolisant alone to 7 T 3.4 when combined with mazindol (n = 1), methylphenidate (n = 1), or sodium oxybate plus modafinil (n = 1). Mean sleep onset latency increased from 31 T 14 minutes to 36 T 8 minutes (P = 0.21) on the maintenance of wakefulness test. The severity and frequency of cataplexy were slightly improved. Adverse effects were minor (insomnia, headache, hot flushes, leg pain, and hallucinations) and transitory, except for insomnia, which persisted in 2 teenagers. The benefit was maintained after a mean of 13 months.

Pitolisant could constitute an acceptable alternative for the treatment of refractory sleepiness in teenagers with narcolepsy.

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Epilepsy is one of the most common brain disorders. Despite the availability of many various antiepileptic drugs, there is still strong need for new compounds. Pharmacological studies have confirmed that histamine plays a role in epilepsy. Although the involvement of histamine H$_3$ receptor in epilepsy is still under consideration, undertaken experimental studies have shown effectiveness of histamine H$_3$ antagonists/inverse agonists in acute and chronic models of epilepsy. Recently published results have shown effective of *pitolisant* in the human photosensitivity model of epilepsy in early phase II studies. This work is a part of our search for potential anticonvulsant agents among histamine H$_3$ receptor ligands. Ether derivatives of (un)substituted (homo)piperidines were tested for their binding affinities at recombinant human histamine H$_3$ receptor and exhibited pronounced to good affinities (K$_i$ values from 42 to 358 nM). Compounds were evaluated for anticonvulsant activity and neurotoxicity according to the standard protocols within Anticonvulsant Screening Program at the NIH/NINDS Bethesda (USA). For some compounds good protection against MES induced seizures was observed. However neurotoxicity was also detected in some cases. Activity of the examined compounds depended on the character of aromatic substituent and length of alkyl chain. The most promising results were obtained for 1-(5-(naphthalen-1-yloxy)pentyl)piperidine.

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EHRS International Anthem
EHRS International Anthem

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-hydroxytryptamine / and up with good old hista,
   Singing Histaminosis all the day

6. Each year we meet in May / to concentrate and play,
   Singing Histaminosis all the day
   What luck to have such friends / to cater for our trends
   Singing Histaminosis all the day

CHORUS

7. In nineteen seventy two / to Paris we all flew,
   Singing Histaminosis all the day.
   Then Marburg upon Lahn / where Wilfried kept us calm,
   Singing Histaminosis all the day.
8. Copenhagen as next year / the Mermaid to cheer,
   Singing Histaminosis all the day.
   In nineteen seventy five / Florence kept us alive,
   Singing Histaminosis all the day.

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

CHORUS

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

11. Then to Budapest we went / with Susan on the scent,
   Singing histaminosis all the day.
   West Germany again / for Hannover by name,
   Singing Histaminosis all the day.

12. In nineteen eighty two / to Bled we all flew,
   Singing Histaminosis all the day.
   Then Brighton to the fore / with sea breezes by the shore,
   Singing Histaminosis all the day.

CHORUS

13. And in nineteen eighty four / back in Florence like before,
   Singing Histaminosis all the day.
   Then in Aachen eighty five / Charlemagne became alive,
   Singing Histaminosis all the day.

14. Then in Odense in Spring / in the Castle we did sing,
   Singing Histaminosis all the day.
   And then Czecho was the next / with our Rado at his best,
   Singing Histaminosis all the day.

15. G.B. West was then cheered / for the ten years we´d been steered,
   Singing Histaminosis all the day.
   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,
   We´re in Holland for the very first time.
   To Kuopio in Finland / to the beautiful, but cold land,
   we were watching the Finnish chopping wood.
17. Then to Marburg we returned / ninety one and also learned
That histamine in surgery’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.
Back to Germany next spring / and with Helmut did we sing
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.
EHRS International Anthem

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

28. Then to Łódź again next year / for the fourth time we meet here!!
Dear Agnieszka both Honorar and chair.
In two thousand and fourteen / Then Lyon was back on scene
And our Lin made it most amazing there
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 submitted to the Publications Secretary (Gill Sturman) by the 1st June at the very 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 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.*
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