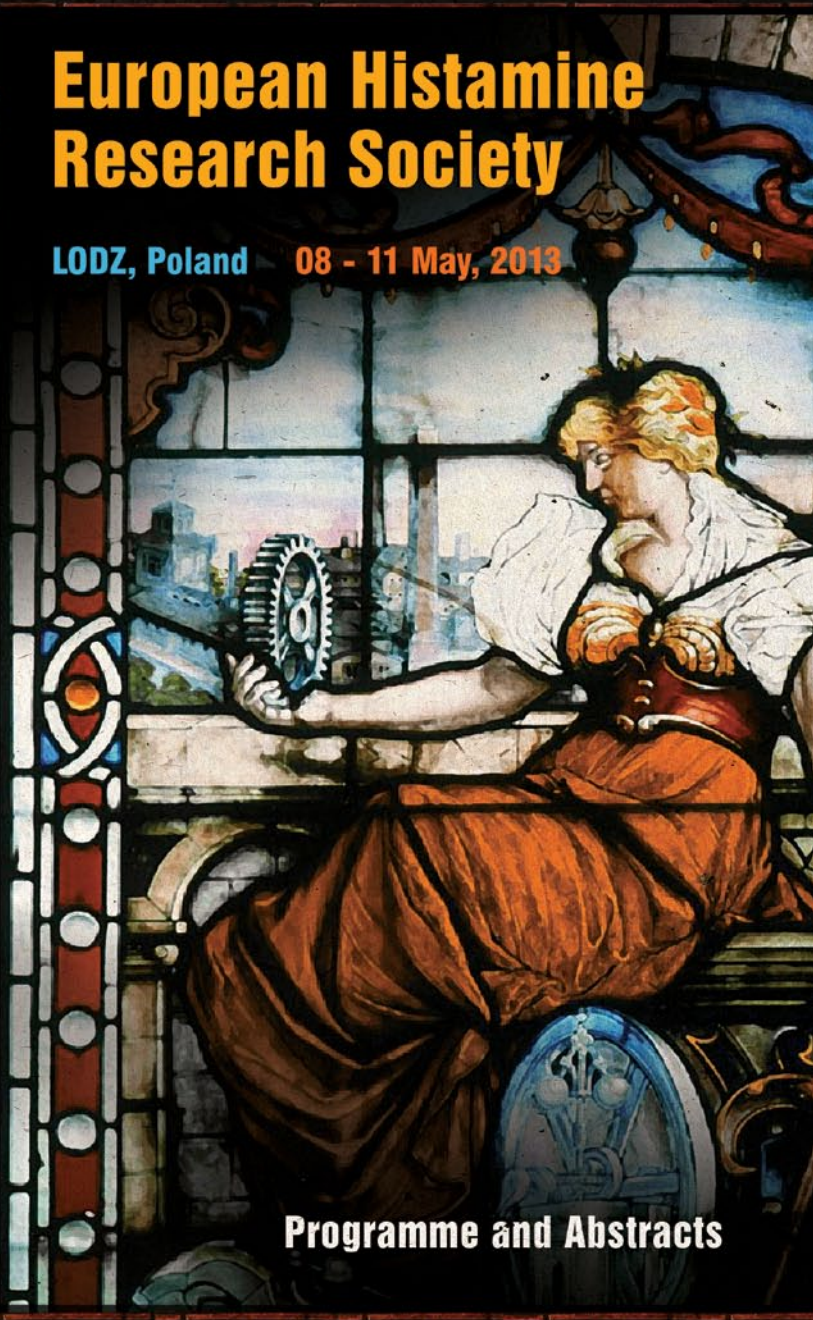
42nd Annual Meeting of the European Histamine Research Society

LODZ, Poland 08 - 11 May, 2013



Programme and Abstracts

Invited Speakers:

Armin Buschauer (Germany)
Marek Jutel (Poland)
Pertti Panula (Finland)
Satoshi Tanaka (Japan)
William Wisden (UK)

The Organisers:

POLISH HISTAMINE RESEARCH SOCIETY

DEPARTMENT OF HORMONE BIOCHEMISTRY
MEDICAL UNIVERSITY OF LODZ

Welcome

On behalf of the Organizing Committee, I would like to welcome you to Lodz for the 42nd meeting of the European Histamine Research Society held in the Ambasador Centrum Hotel, in the centre of the City. As usual, the meeting will begin with the Get-together Party on Wednesday 8nd May and end with breakfaston Sunday 12th May. Likewise, the conference will cover our usual wide range of interesting topics: histamine and all of the histamine system aspects, from medicinal chemistry, pharmacology, physiology, biochemistry, behaviour to pathophysiology i.e., histamine receptors, metabolism, transport, histamine containing cells (mast cells, basophils, neurons, etc), interaction of histamine system with other signalling systems.

Presenters of new results from basic and clinical research are welcome, as well as those who will just listen, learn and discuss. An interesting partner programme is provided for accompanying persons. After long hours spent in the conference room, we all will have good opportunities to recover and network during attractive social sessions, scheduled each day in the evening.

Do not forget, Lodz will be hosting histaminologists for the fourth time already; the previous meetings were in 1978 and 1998, not to mention the first informal one, in August 1971, the satellite symposium to XXVth International Congress of the Physiological Society, the symposium related to our unique amine. The remarkable outcomes of this symposium were the "Histamine Club" and a book entitled "Histamine" edited by Czeslaw Maslinski –the Organiser, and published by Dowden, Hutchinson and Ross Inc., Stroudsburg, PA, 1974. Lodz has a rather long history; established as a city in 1423, was still very small (700 inhabitants) in XVI century. The abrupt change occurred in XIX century when it was decided to set up a textile industry there. German, Jews, Russians and Czechs with the experience and money came to manufacture and trade cotton, wool, fabrics and cloth. They built up factories, houses and palaces. The City developed very fast; within 100 years Lodz from the town of sixty thousand inhabitants grew up to six hundred thousand before the 2nd World War. Lodz prides itself with both a beautiful and the biggest collection of Art Nouveau buildings in Europe. The most attractive mill owned by Poznanski has recently been renewed, new modern wings were attached and under the name "Manufactura" it serves as a trade, cultural and social center with shops, museums, cinemas, restaurants.

You are cordially welcome, I am sure you will enjoy both, the EHRS Meeting and the City.

W. Agnieszka Fogel

Previous EHRS Annual Meetings

1971 Lodz
1972 Paris
1973 Marburg
1974 Copenhagen
1975 Florence
1976 Paris
1977 London
1978 Lodz
1979 Stockholm
1980 Visegrad
1981 Hannover
1982 Bled
1983 Brighton
1984 Florence
1985 Aachen
1986 Odense
1987 Strbske Pleso
1988 Copenhagen
1989 Breda
1990 Kuopio
1991 Marburg
1992 Malaga
1993 Cologne
1994 Budapest
1995 Moscow
1996 Antwerp
1997 Seville
1998 Lodz
1999 Lyon
2000 Nemi (Rome)
2001 Turku
2002 Eger
2003 Noordwijkerhout
2004 Bergisch-Gladbach
2005 Bled
2006 Delphi
2007 Florence
2008 Stockholm
2009 Fulda
2010 Durham
2011 Sochi
2012 Belfast

42nd Annual Meeting, 8-11 May 2013, Lodz, Poland

Committees

Chairman of the meeting: W. Agnieszka Fogel

Scientific & Organizing Committee

W. Agnieszka Fogel (Lodz, Poland) - chairman
Anna Stasiak (Lodz, Poland)
Jerzy Jochem (Bytom, Poland)
Katarzyna Kieć-Kononowicz (Cracow, Poland)
Miroslaw Mussur (Lodz, Poland)
Krystyna Sasiak (Lodz, Poland)
Barbara Skrzydło-Radomańska (Lublin, Poland)
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International Advisory Committee

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Holger Stark (Frankfurt am Main, Germany)
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The EHRS Young Investigator Award Committee

Anita Sydbom (Stockholm, Sweden) - chairman
Astrid Sasse (Dublin, Ireland)
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Poster Prize Committee

Jerzy Jochem (Bytom, Poland) - chairman
M. Beatrice Passani (Firenze, Italy)
Helmut L. Haas (Düsseldorf, Germany)
Hiroyuki Fukui (Tokushima, Japan)
Pertti Panula (Helsinki, Finland)
Dariusz Matosiuk (Lublin, Poland)
Holger Stark (Frankfurt am Main, Germany)
Nicholas Carruthers (San Diego, USA)
Krzysztof Walczyński (Lodz, Poland)
Andras Falus (Budapest, Hungary)
Katarzyna Kieć-Kononowicz (Cracow, Poland)
Fred Pearce (Brighton, UK)
Madeleine Ennis (Belfast, UK)
Dariusz Szukiewicz (Warsaw, Poland)

Abstract Evaluation & Bursary Committees

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Anita Sydbom (Stockholm, Sweden)
Pertti Panula (Helsinki, Finland)
Gill Sturman (Essex, UK)
Nicholas Carruthers (San Diego, USA)
Jian-Sheng Lin (Lyon, France)
M. Beatrice Passani (Firenze, Italy)
Astrid Sasse (Dublin, Ireland)
Pierre Chatelain (Bruxelles, Belgium)

CONFERENCE PROGRAMME

THE PROGRAMME AT A GLANCE

Wednesday, May 8th 2013

15.00 – 20.00	Registration, Ambasador Centrum Hotel
16.00 – 19.00	Poster mounting Ambasador Centrum Hotel, Conference room "A"
16.00 – 18.00	EHRS Council Meeting Ambasador Centrum Hotel, Conference room "D"
19.00 – 22.00	Welcome reception Ambasador Centrum Hotel, Restaurant

Thursday, May 9th 2013

8.30 – 9.00	**Opening Ceremony** Presentation of Bursary Award Winners
9.00 – 9.45	**Honorary Membership Ceremony** **Wiesława Agnieszka Fogel (Poland)** – introduced by Wilfried Lorenz
9.45 – 10.30	*Invited Lecture* **Pertti Panula** (University of Helsinki, Finland): **Histamine and addiction: from behavior to neurotransmitter interactions**
10.30 – 11.00	Coffee break
11.00 – 12.45	**HISTAMINE IN CNS, part I** **Oral and poster presentations**
13.00 – 14.00	Lunch
14.00	Trip to Częstochowa Dinner, GOLD MILL Inn, KRUSZÓW Transfer back

Friday, May 10th 2013

8.30 – 9.35	**HISTAMINE IN CNS, part II**
8.30 – 9.15	*Invited Lecture* **William Wisden** (Imperial College London, UK): **Histamine at the intersection of circadian rhythms and the sleep-wake cycle?**
9.35 – 12.00	**HISTAMINE RECEPTORS SESSION, part I**
9.35 – 10.20	*Invited Lecture* **Armin Buschauer** (University of Regensburg, Germany): **Toward selective molecular tools for histamine H_2 and H_4 receptors: conformational constraints, bioisosteric and bivalent approaches**
10.20 – 10.45	Coffee break and poster viewing

10.45 – 12.00	Oral and poster presentations
12.00 – 13.30	Lunch
13.30 – 15.15	**HISTAMINE RECEPTORS (part II) AND METHODS** Oral and poster presentations
15.15 – 15.45	Coffee break and poster viewing
15.45 – 16.45	**HISTAMINE AND CANCER** Oral and poster presentations
17.00	Sandwich/Coffee/Tea (Hall, next to the conference room)
18.00	Bus transfer to the Grand Theater Lodz
18.30	Ballet "Promised Land", the Grand Theater Lodz Composer: Gray Veredon/Franz von Suppé and Michael Nyman Author of the libretto: Gray Veredon, inspired by Władysław Reymont's novel and a movie by Andrzej Wajda
21.30	Dinner Ambasador Centrum Hotel, Restaurant

Saturday, May 11th 2013

9.00 – 11.00	**HISTAMINE AND CELLS, part I**
9.00 – 9.45	*GB West lecture* **Satoshi Tanaka** (Okayama University, Japan): **Histamine synthesis and its functions in murine mast cells**
10.00 – 11.00	Oral and poster presentations
11.00 – 11.30	Coffee break and poster viewing
11.30 – 13.25	**HISTAMINE AND CELLS, part II**
11.30 – 12.15	*Invited Lecture* **Marek Jutel** (Wroclaw Medical University, Poland): **The role of histamine signalling in pathomechanism of non-specific IBD**
12.15 – 13.30	Oral and poster presentations
13.30 – 14.15	Lunch and poster viewing
14.15 – 14.35	**HISTAMINE DATABASES' PRESENTATION**
14.35 – 16.05	**YOUNG INVESTIGATOR AWARD SYMPOSIUM**
16.05 – 16.30	Coffee break
16.30 – 18.00	**General Assembly of the EHRS**
19.30 – 00.00	**Farewell Dinner** Ambasador Centrum Hotel, Restaurant

SOCIAL PROGRAMME

Wednesday, May 8th 2013

19.00 – 22.00 **Welcome reception**
Ambasador Centrum Hotel, Restaurant

Thursday, May 9th 2013

14.00 **Trip to Częstochowa**
JASNA GÓRA - Shrine and Monastery

The tour includes the Knights' Hall, the 600th Year Anniversary of Jasna Góra Museum, the St. Roch Bastion, and the Treasury, as well the Chapel of Miraculous Painting of Our Lady and the Basilica of Jasna Góra (http://www.jasnagora.pl).

Due to the sacred character of the place, suitable clothes are required. Comfortable shoes might be useful.

20.30 **Dinner - GOLD MILL Inn, KRUSZÓW**

Friday, May 10th 2013

18.00 **Bus transfer to the Grand Theater Lodz**

18.30 **Ballet "Promised Land", the Grand Theater Lodz**
Composer: Gray Veredon / Franz von Suppé and Michael Nyman
Author of the libretto: Gray Veredon, inspired
by Władysław Reymont's novel and a movie by Andrzej Wajda

In 1999 Gray Veredon, a British choreographer fascinated with the history of the city of Lodz created a unique ballet show. He looked at its residents and development of social relations at the turn of the 20th century from his own perspective and dedicated his production to Lodz: to the past and the present of the city.
The production was inspired by a novel written by Władysław Reymont and a film adaptation of the novel directed by Andrzej Wajda. The action takes place at the end of 19th century. A group of three friends: a Pole Karol Borowiecki, a German Max Baum and a Jew Moritz Welt, decide to settle in Lodz and set up a textile factory.
Veredon created a faithful adaptation of the novel and preserved the most important themes created by Reymont. It shows the story of Anka, hopelessly in love with Karol, his affair with Lucy Zucker, intrigues of the competition, and the character of crude Müller. But the main protagonist of the play is the city itself and, most of all, the silent creators of its power, the laborers of Lodz: weavers and dyers. Promised Land is a deeply moving show about the cultural identity of Lodz (Source: http://www.operalodz.com)

21.30 **Dinner (Ambasador Centrum Hotel, Restaurant)**

Saturday, May 11th 2013

19.30 – 00.00 **Farewell Dinner**
Ambasador Centrum Hotel, Restaurant

ACCOMPANYING PARTNERS PROGRAMME

Wednesday, May 8th 2013

19.00 – 22.00	**Welcome reception** Ambasador Centrum Hotel, Restaurant

Thursday, May 9th 2013

8.30 – 9.00	**Opening Ceremony**
9.00 – 9.45	**Honorary Membership Ceremony** **Wiesława Agnieszka Fogel (Poland)** – introduced by Wilfried Lorenz
10.00	**Visit the Museum of Cinematography - Palace of Karol Scheibler** (Meeting point: Reception Hall, Ambasador Centrum Hotel)

The palace received its final neo-Renaissance shape in 1888 (designed by E. Lilpop). Calm, balanced facade contrasts with the wealth of interiors maintained in multiple styles. Stucco decorations, painted decorations, furniture, fireplaces, furnaces, paneling, fabric for furniture covering and Venetian mosaics leave an unforgettable impression. The Museum collects exhibits related to the history of the Polish cinematography. The permanent exhibition presents devices associated with the development of film technique. Temporary exhibitions are dedicated to the history of film, contemporary Polish super-productions, as well as Polish photography and art of the media.

13.00 – 14.00	**Lunch**
14.00	**Trip to Częstochowa** Dinner, GOLD MILL Inn, KRUSZÓW Transfer back

Friday, May 10th 2013

10.00	**LODZ TOUR** (Meeting point: Reception Hall, Ambasador Centrum Hotel) Lunch at Manufaktura

Piotrkowska Street *is the representative street of Lodz. One of the longest shopping streets in Europe has a length of approximately 4.2 km and runs longitudinally in a straight line, between Plac Wolności and Plac Niepodległości. From the beginning, this street was a central axis, around which the city expanded, and its development spontaneously gave the city center its current shape. Initially, the street was mainly a communication route, but with time, it became the "city's visiting card", a center of entertainment and commerce, a focus point of the entire life of the growing industrial agglomeration. When visiting "Pietryna" (Piotrkowska Str.), one can look at the beautiful facades of the Art Nouveau buildings and see the monuments of, for example, Three Manufacturers, Tuwim's Bench or Monument of Citizens of Lodz at the Turn of the Millennium - 12,859 blocks with engraved names.*

Palace of Izrael K. Poznański / Museum of the City of Lodz *is the largest manufacturer's residence in Poland. There are various styles to be found in its architecture. An Art Nouveau staircase leads to a neo-baroque dinning room and a ballroom. The Palace is home to Museum of the City of Lodz, in which one can become familiar with*

the history and culture of the 19th century industrial metropolis. The permanent exhibition "The Pantheon of the Famous Citizens of the City of Lodz" presents the profiles of prominent artists associated with the city: Wł. Reymont, J. Tuwim, J. Kosiński, K. Dedecius and A. Tansman. The memorabilia left after A. Rubinstein were assembled in the world's only gallery dedicated to the famous pianist.

***Manufaktura** is a center of entertainment, culture and commerce. A revitalization project, unique at the Polish and European scale, combining modern forms and architecture with the restored 19th century buildings of the former factory of Izrael Poznański. 20 ha of surface include, among others, a Market with colorful fountains - a place, which hosts festivals, concerts and outdoor events, Museum of the Factory, ms2 Museum of Art, restaurants, over 300 shops, discos, a bowling alley, a climbing wall and a multiplex cinema.*

***Museum of Art** in Lodz is one of the oldest museums of a modern art in the world. The Museum's connections with the avant-garde date back to the turn of the 1920s and 1930s, when a group of radical artists from the "a.r." group began gathering works of the most important artists of the day for the Museum. The action met with great interest of European avant-garde, making many outstanding artists, such as Fernand Leger, Max Ernst, Hans Arp and Kurt Schwitters to donate their works to the collection. The fact made the collection of the "a.r." group a unique symbol of solidarity and cooperation of the avant-garde. The International Modern Art. Collection of the "a.r." group, representing the main directions of art. such as Cubism, Futurism, Constructivism, Purism, Neoplasticism and Surrealism was open to public on 15th February 1931. The collection has since been consistently expanded by including international modern and contemporary artworks. Due to this fact Museum of Art is now the only museum in Poland with such extensive collection of world art of the 20th and 21st century.*

Source: www.turystyczna.lodz.pl, http://www.msl.org.pl

17.00	**Sandwich/Coffee/Tea** Hall, next to the conference room
18.00	**Bus transfer to the Grand Theater Lodz**
18.30	**Ballet "Promised Land", the Grand Theater Lodz** Composer: Gray Veredon/Franz von Suppé and Michael Nyman Author of the libretto: Gray Veredon, inspired by Władysław Reymont's novel and a movie by Andrzej Wajda
21.30	**Dinner** Ambasador Centrum Hotel, Restaurant

Saturday, May 11th 2013

10.30	**Visit the Central Museum of Textiles - Ludwik Geyer's White Factory** (Meeting point: Reception Hall, Ambasador Centrum Hotel)

The seat of the Museum is the White Factory, a magnificent complex of classicist buildings, one of the most beautiful monuments of industrial architecture in Poland. Erected by Ludwig Geyer in 1835-1839, the White Factory (many times expanded and re-built) was the first in Poland multi-department factory. It housed the first in Poland mechanical spinning, weaving and printing rooms for cotton, driven by steam machine (as a consequence of steam machine work, the first chimney was erected nearby the factory premises, the first one in Lodz; later, Lodz was often referred to as "the city of chimneys"). There we can see the tools, textile machinery, historical and contemporary textiles, as well as clothing. The Museum is the organizer of International Triennal of the Tapestry. Within the museum, there is Open-Air Museum of the Lodz Wooden Architecture, presenting an example of the city's buildings in the early 19th century.

13.30 – 14.15	**Lunch** Ambasador Centrum Hotel, Restaurant
19.30 – 00.00	**Farewell Dinner** Ambasador Centrum Hotel, Restaurant

SCIENTIFIC PROGRAMME

Wednesday, May 8th 2013

15.00 – 20.00	Registration, Ambasador Centrum Hotel
16.00 – 19.00	Poster mounting Ambasador Centrum Hotel, Conference room "A"
16.00 – 18.00	EHRS Council Meeting, Conference room "D"
19.00 – 22.00	Welcome reception Ambasador Centrum Hotel, Restaurant

Thursday, May 9th 2013

8.30 – 9.00	**Opening Ceremony** Presentation of Bursary Award Winners
9.00 – 9.45	**Honorary Membership Ceremony** **Wiesława Agnieszka Fogel (Poland)** – introduced by Wilfried Lorenz
9.45 – 10.30 *Invited Lecture*	**PERTTI PANULA** *(introduction by M.B. Passani)* **HISTAMINE AND ADDICTION: FROM BEHAVIOR TO NEUROTRANSMITTER INTERACTIONS** P. Panula, J. Vanhanen, T. Mäki, S. Rozov, S. Nuutinen *Neuroscience Center and Institute of Biomedicine, Anatomy, University of Helsinki, Finland*
10.30 – 11.00	*Coffee break and poster viewing*
11.00 – 12.45	**HISTAMINE IN CNS, part I** **Chaired by: M.B. Passani and H.L. Haas**
11.00 – 11.15 *Oral presentation*	**HISTAMINERGIC NEURONS ARE EXITED BY PROTONS** H.L. Haas, Y. Yanovsky, A. Kernder, O.A. Sergeeva *Department of Neurophysiology, Heinrich-Heine-University, Düsseldorf, Germany*
11.15 – 11.30 *Oral presentation*	**ABT-239, AN H_3 RECEPTOR ANTAGONIST/INVERSE AGONIST, INCREASES HISTAMINE RELEASE AND C-FOS EXPRESSION IN BRAIN REGIONS INVOLVED IN MEMORY AND COGNITION** G. Provensi, L. Munair, M.B. Passani, P. Blandina *Dipartimento di Neuroscienze, dell'Area del Farmaco e della Salute del Bambino - NEUROFARBA, Universitá di Firenze, Firenze, Italy*
11.30 – 11.45 *Oral presentation*	**BEHAVIOURAL EVIDENCE FOR CENTRAL H_4 HISTAMINE RECEPTORS** R.M.A. Abuhamdah, M.A. Katebe, A. Ennaceur, P.L. Chazot *School of Biological & Biomedical Sciences, Durham University, UK; Sunderland Pharmacy School, UK*

11.45 – 12.00
Oral presentation

THE HISTAMINE H_4 RECEPTOR IS FUNCTIONALLY EXPRESSED ON RAT SUBSTANCE P-CONTAINING DORSAL ROOT GANGLIA
M.A. Katebe and P.L. Chazot
School of Biological & Biomedical Sciences, Durham University, UK

12.00 – 12.15
Oral presentation

HISTAMINE TRANSPORT BY MOUSE PLASMA MEMBRANE MONOAMINE TRANSPORTER AND MOUSE ORGANIC CATION TRANSPORTER 3
F. Naganuma, T. Yoshikawa, T. Nakamura, T. Iida, R. Harada, A. Mohsen, K. Yanai
Tohoku University Graduate School of Medicine, Department of Pharmacology, Sendai, JAPAN

12.15 – 12.20
P1

PHENYTOIN DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS IN EPILEPSY MODELS IN RATS
B. Sadek, M. Walter, A. Adem, S. Shehab, S. Dhanasekaran, M. Shafiullah, L. Weizel, J.S. Schwed, H. Stark
Department of Pharmacology and Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, UAE; Biocenter, Institute of Pharmaceutical Chemistry, Johann Wolfgang Goethe University, Frankfurt, Germany

12.25 – 12.30
P2

THE HYPOPHAGIC FACTOR OLEOYLETHANOLAMIDE RECRUITES HYPOTHALAMIC HISTAMINE PATHWAYS TO INHIBIT FOOD INTAKE
H. Umehara, G. Provensi, L. Canto-de-Souza, R. Fabbri, P. Blandina, M.B. Passani
Dipartimento di Farmacologia Preclinica e Clinica, Universitá di Firenze, Firenze, Italy

12.30 – 12.45
Oral presentation

CARDIOVASCULAR EFFECTS OF HISTAMINE H_3 RECEPTOR INVERSE AGONISTS IN HAEMORRHAGE-SHOCKED RATS
J. Jochem
Department of Basic Medical Sciences, Medical University of Silesia, Bytom, Poland

13.00 – 14.00

Lunch

14.00

Trip to Częstochowa
Dinner, GOLD MILL Inn, KRUSZÓW
Transfer back

Friday, May 10th 2013

8.30 – 9.35

HISTAMINE IN CNS, part II
Chaired by: H. Fukui and P. Panula

8.30 – 9.15
Invited Lecture

WILLIAM WISDEN *(introduction by H.L. Haas)*
HISTAMINE AT THE INTERSECTION OF CIRCADIAN RHYTHMS AND THE SLEEP-WAKE CYCLE?
Department of Life Sciences, Imperial College London, UK

9.15 – 9.30
Oral presentation

ENHANCED HISTAMINERGIC NEUROTRANSMISSION AND SLEEP-WAKE ALTERATIONS, A STUDY IN HISTAMINE H_3-RECEPTOR KNOCK-OUT MICE
E. Gondard, C. Anaclet, H. Akaoka, R. Guo, M. Zhang C. Buda, P. Franco, H. Kotani, J.S. Lin
Integrative Physiology of the Brain Arousal Systems, Lyon Neuroscience Research Center, INSERM, Faculty of Medicine, Claude Bernard University, Lyon, France

9.30 – 9.35
P4

HISTAMINERGIC TUBEROMAMILLARY NUCLEUS CONSTITUTES ONE OF THE MOST IMPORTANT TARGETS FOR THE WAKE-PROMOTING EFFECT OF OREXIN NEURONS BUT NOT THE EXCLUSIVE ONE

C. Anaclet, Y. Zhao, H.L. Haas, J.S. Lin

Integrative Physiology of the Brain Arousal Systems, Lyon Neuroscience Research Center, INSERM, Faculty of Medicine, Claude Bernard University, Lyon, France

9.35 – 12.00

HISTAMINE RECEPTORS, part I
Chaired by: D. Matosiuk and H. Stark

9.35 – 10.20
Invited Lecture

ARMIN BUSCHAUER *(introduction by H. Stark)*
TOWARD SELECTIVE MOLECULAR TOOLS FOR HISTAMINE H_2 AND H_4 RECEPTORS: CONFORMATIONAL CONSTRAINTS, BIOISOSTERIC AND BIVALENT APPROACHES

A. Buschauer, R. Geyer, P. Igel, P. Baumeister, T. Birnkammer, D. Erdmann, U. Nordemann, N. Kagermeier, T. Holzammer, D. Wifling, J. Felixberger, D. Schnell, I. Brunskole, K. Ladova, K. Löffel, T. Reher, D. Neumann, Strasser, G. Bernhardt, R. Seifert, S. Dove

Institute of Pharmacy, University of Regensburg, Regensburg, Germany; Institute of Pharmacology, Medical School of Hannover, Hannover, Germany

10.20 – 10.45

Coffee break and poster viewing

10.45 – 11.00
Oral presentation

HISTAMINE H_3 RECEPTOR ACTIVITY OF (HOMO)PIPERIDINYL-PENTOXYPHENYL DERIVATIVES

K. Kieć-Kononowicz, S. Schwed, L. Weizel, H. Stark, D. Łażewska

Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Institute of Pharmaceutical Chemistry, Goethe University, Biozentrum, ZAFES/CMP/ICNF, Frankfurt/Main, Germany

11.00 – 11.15
Oral presentation

NON-IMIDAZOLE HISTAMINE H_3 LIGANDS. SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVESTIGATION OF 1-[2-THIAZOL-5-YL- AND 1-[2-THIAZOL-4-YL-(2-AMINOETHYL)]-4-N-PROPYLPIPERAZINE DERIVATIVES

M. Staszewski, R. Guryn, K. Walczyński

Department of Synthesis and Technology of Drugs, Medical University of Lodz, Lodz, Poland

11.15 – 11.20
P11

BENZYLPIPERIDINE VARIATIONS ON HISTAMINE H_3 RECEPTOR ANTAGONISTS FOR IMPROVED DRUGLIKENESS

K. Wingen, K. Isensee, J.S. Schwed, L. Weizel, A. Živković, D. Odazic, H. Stark

Johann Wolfgang Goethe University, Institute of Pharmaceutical Chemistry, ZAFES/CMP/ICNF, Biozentrum, Frankfurt/Main, Germany

11.20 – 11.25
P12

AMIDE DERIVATIVES OF 4-METHYLPIPERAZIE AS HISTAMINE H_4 RECEPTOR LIGANDS

D. Łażewska, J. Cezary, S. Schwed, L. Weizel, H. Stark, K. Kieć-Kononowicz

Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Institute of Pharmaceutical Chemistry, Goethe University, Biozentrum, ZAFES/CMP/ICNF, Frankfurt/Main, Germany

11.25 – 11.30
P13

SEARCH FOR NEW HISTAMINE H4 RECEPTOR LIGANDS IN THE GROUP OF 1,3,5-TRIAZINE DERIVATIVES

M. Stelmasiński, P. Porwisz, M. Więcek, K. Kamińska, T. Kottke, S. Schwed, J. Handzlik, H. Stark, K. Kieć-Kononowicz

Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland; Institute of Pharmaceutical Chemistry, Johann Wolfgang Goethe University, Frankfurt, Germany

11.30 – 11.35
P14

***IN VITRO* CYTOTOXICITY EVALUATION OF 3 PIPERIDINOPROPAN-1-OL DERIVATIVES WITH H_3R ACTIVITY AND 1,3,5-TRIAZINE DERIVATIVES WITH H_4R ACTIVITY**

G. Latacz, K. Bąk, D. Łażewska, K. Kieć-Kononowicz

Jagiellonian University, Medical College, Department of Technology and Biotechnology of Drugs, Cracow, Poland

11.35 – 11.40
P15

PIPERAZINE MODIFICATION IN 2,4,6-TRIAMINOPYRIMIDINE DERIVATIVES AS HISTAMINE H_4 RECEPTOR LIGANDS

A. Schreeb, M. Walter, D. Odadzic, J. S. Schwed, L. Weizel, H. Stark

Goethe University, Institute of Pharmaceutical Chemistry, Biocenter, Frankfurt, Germany

11.40 – 12.00
Oral presentation

HISTAMINE H_4 RECEPTOR DRUG DISCOVERY: LESSONS LEARNED AND NEW OPPORTUNITIES

R.A. Smits, H.D. Lim, M. Andaloussi, T. van der Meer, I.J.P. de Esch, R. Leurs

Griffin Discoveries BV, Amsterdam, The Netherlands

12.00 – 13.30

Lunch

13.30 – 15.15

HISTAMINE RECEPTORS, part II AND METHODS
Chaired by: N. Carruthers and K. Walczyński

13.30 – 13.50
Oral presentation

DISCOVERY OF JNJ 39758979 AND SAR OF RELATED 2-AMINOPYRIMIDINE HISTAMINE H_4 ANTAGONISTS

B.M. Savall

Janssen Research & Development, L.L.C., San Diego, USA

13.50 – 14.05
Oral presentation

THE HISTAMINE 4 RECEPTOR INHIBITS HUMAN NEUTROPHIL PHAGOCYTOSIS

K. Dib, T. Scheithauer, T. Perecko, V. Jenei, R. L. Thurmond, M. Ennis

Centre for Infection and Immunity, Queen's University of Belfast, Belfast, United Kingdom; Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia; Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i, Brno, Czech Republic; Cancer Sciences Unit, University of Southampton, United Kingdom; Janssen Research & Development, L.L.C. San Diego, CA, USA

14.05 – 14.20
Oral presentation

JNJ7777120 COMPOUND AMELIORATES DAMAGE IN SALIVARY GLANDS, GINGIVA AND PERIODONTAL BONE PRODUCED BY EXPERIMENTALLY INDUCED PERIODONTITIS IN RATS

J.P. Prestifilippo, J. Fernández-Solari, D. Martinel Lamas, C. Mohn, E.S. Rivera, J.C. Elverdin, V.A. Medina

Physiology Department, School of Dentistry, University of Buenos Aires, Argentina; Physiopathology Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Argentina; Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

14.20 – 14.25
P16

THE EFFECTS OF THE H_4R ANTAGONIST JNJ7777120 ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY HUMAN NEUTROPHILS

T. Perecko, K. Drabikova, R. Nosal, V. Jancinova
Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic; Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

14.25 – 14.30
P17

EFFECTS OF HISTAMINE AND ITS H_4 RECEPTOR AGONISTS ON REACTIVE OXYGEN SPECIES PRODUCTION IN HUMAN LEUKOCYTES

O. Vasicek, M. Ciz, A. Lojek
Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic; Masaryk University, Faculty of Science, Brno, Czech Republic

14.30 – 14.35
P18

ANTINOCICEPTIVE ACTION OF JNJ7777120 IN ACUTE PAIN MODELS AND ITS INTERACTIONS WITH ARACHIDONIC ACID DERIVATIVES INHIBITORS

P. Rzodkiewicz, E. Gąsińska, D. Łażewska, M. Bujalska-Zadrożny, D. Maślińska, K. Kieć-Kononowicz, E. Wojtecka-Łukasik, D. Szukiewicz, S. Maśliński
Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland

14.35 – 14.50
Oral presentation

THE ROLE OF HISTAMINE RECEPTORS ON GLUCAGON SECRETION IN ATC1.6 CELL

T. Nakamura, T. Yoshikawa, F. Naganuma, R. Harada, A. Mohsen, K. Yanai
Tohoku University Graduate School of Medicine, Department of Pharmacology, Sendai, Japan

14.50 – 14.55
P19

EXPRESSION OF HUMAN HISTAMINE H_2-RECEPTORS IN TRANSGENIC MICE

J. Neumann, F. Köhler, J. Meister, U. Kirchhefer, U. Gergs
Institut für Pharmakologie und Toxikologie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, Halle/Saale, Germany; Institut für Pharmakologie und Toxikologie, Westfälische Wilhelms-Universität Münster, Münster, Germany

14.55 – 15.10
Oral presentation

CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR HUMAN HISTAMINE N-METHYLTRANSFERASE

H.G. Schwelberger, J. Feurle, G. Houen
Molecular Biology Laboratory, Dep. Visceral, Transplant & Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria; Statens Serum Institut, Copenhagen, Denmark

15.10 – 15.15
P20

EOSINOPHIL PURIFICATION FROM PERIPHERAL BLOOD – STUDY OF DIFFERENT IMMUNOMAGNETIC CELL SORTING METHODS EFFICIENCY

M. Grosicki, T. Karcz, K. Bukowska-Strakova, K.Kieć-Kononowicz
Jagiellonian University Medical College, Faculty of Pharmacy, Department of Technology and Biotechnology of Drugs, Cracow, Poland; Jagiellonian Centre for Experimental Therapeutics (JCET), Cracow, Poland

15.15 – 15.45 *Coffee break and poster viewing*

15.45 – 16.45 **HISTAMINE AND CANCER**
Chaired by: A. Falus and J. Jochem

15.45 – 16.00
Oral presentation

RADIOPROTECTIVE EFFECT OF JNJ7777120 AGAINST CYTOTOXIC AND GENOTOXIC DAMAGE OF IONIZING RADIATION

D. Martinel Lamas, E. Carabajal, J.E. Cortina, P. Ciraolo, E. Rhon Calderón, S. Merani, E.S. Rivera, V.A. Medina
Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; Patagonic National Center – CONICET, Chubut, Argentina; Center of Reproduction Research, School of Medicine, University of Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Argentina

16.00 – 16.05
P5

NORMAL FIBROBLASTS INDUCE A MESENCHYMAL PHENOTYPE IN MAMMARY EPITHELIAL TUMOR CELLS WHICH IS BLOCKED BY HISTAMINE TREATMENT OF FIBROBLASTS

J. Porretti, N. Mohamad, E. Badenas, M. Esnaola, Micaela Santoro, D. Martinel Lamas, G. Martín, G. Cricco
Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

16.05 – 16.10
P6

ANTIPROLIFERATIVE EFFECTS OF H_4R AGONISTS ON HUMAN WIDR COLORECTAL CANCER CELL LINE

P. Ciraolo, D. Martinel Lamas, J.E. Cortina, E.S. Rivera, V.A. Medina
Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Argentina

16.10 – 16.15
P7

H_4R AGONISTS SUPPRESS HUMAN MELANOMA GROWTH AND LUNG METASTASES

N.A. Massari, V.A. Medina, G.P. Cricco, D. Martinel Lamas, L. Sambuco, P. Ciraolo, J.E. Cortina, E.S. Rivera
Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Argentina; Institute of Immunooncology, Buenos Aires, Argentina

16.15 – 16.20
P8

EXPRESSION PATTERN OF HISTAMINE-RELATED GENES IN ENDOMETRIAL CANCER

A. Jęda, G. Janikowska, G. Cwynar, M. Ciałoń, T. Janikowski, J. Orchel, U. Mazurek, A. Witek
Department and Clinic of Gynecology and Obstetrics, Department of Analytical Chemistry, Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

16.20 – 16.25
P9

COMPARISON OF GENE EXPRESSION PROFILES CONNECTED WITH PROLIFERATION PROCESS IN NORMAL AND ENDOMETRIAL CANCER CELLS

G. Cwynar, A. Jęda, G. Janikowska, M. Ciałoń, T. Janikowski, J. Orchel, U. Mazurek, A. Witek
Department and Clinic of Gynecology and Obstetrics, Department of Analytical Chemistry, Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

16.25 – 16.30
P10

TGF-B GENES INVOLVED IN CELL CYCLE REGULATION IN ENDOMETRIAL CANCER

M. Ciałoń, A. Jęda, G. Janikowska, T. Janikowski, J. Orchel, G. Cwynar, A. Witek, U. Mazurek
Department and Clinic of Gynecology and Obstetrics, Department of Analytical Chemistry, Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

16.30 – 16.45
Oral presentation

AMINOOXY ANALOGUE OF HISTAMINE IS AN EFFICIENT INHIBITOR OF MAMMALIAN L-HISTIDINE DECARBOXYLASE: COMBINED IN SILICO AND EXPERIMENTAL EVIDENCES

R. Castro-Oropeza, A. Pino-Ángeles, M.A. Khomutov, J.L. Urdiales, A.A. Moya-García, A. Khomutov, F. Sánchez-Jiménez
Department of Molecular Biology and Biochemistry, University of Malaga and Unit 741 "Centro de Investigación en Red en EnfermedadesRaras" (CIBERER), Málaga , Spain; Engelhardt Institute of Molecular Biology, Moscow, Russia

17.00 — ***Sandwich/Coffee/Tea*** (Hall, next to the conference room)

18.00 — **Bus transfer to the Grand Theater Lodz**

18.30 — **Ballet "Promised Land", the Grand Theater Lodz**
Composer: Gray Veredon/Franz von Suppé and Michael Nyman
Author of the libretto: Gray Veredon, inspired by Władysław Reymont's novel and a movie by Andrzej Wajda

21.30 — **Dinner**
Ambasador Centrum Hotel, Restaurant

Saturday, May 11th 2013

9.00 – 11.00 — **HISTAMINE AND CELLS, part I**
Chaired by: K. Kieć-Kononowicz and F. Pearce

9.00 – 9.45
Invited Lecture

GB West lecture
SATOSHI TANAKA *(introduction by W.A. Fogel)*
HISTAMINE SYNTHESIS AND ITS FUNCTIONS IN MURINE MAST CELLS
Department of Immunobiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

10.00 – 10.15
Oral presentation

CLINICAL SIGNIFICANCE OF HISTAMINE H_1 RECEPTOR-PKC DELTA-HSP90 SIGNALING IN ALLERGIC SYMPTOMS
H. Fukui, H. Mizuguchi, Y. Kitamura, N. Takeda
Department of Molecular Pharmacology, Department of Otolaryngology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

10.15 – 10.30
Oral presentation

EFFECTS OF A SELECTIVE INHIBITOR POLY (ADP-RIBOSE) POLYMERASE (PARP) ON MAST CELL ACTIVATION BRONCHO-CONSTRICTION AND LUNG FIBROSIS
L. Lucarini, A. Pini, C. Lanzi, D. Bani, R. Pellicciari, F. Moroni, E. Masini
Departments of NEUROFARBA, Section of Pharmacology, Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence; Chemistry and Drug Technology, University of Perugia, Perugia, Italy

10.30 – 10.45
Oral presentation

EVIDENCE FOR "NATURAL" AND "INDUCED" MURINE BASOPHILS DIFFERING IN CALCIUM-DEPENDENT RESPONSIVENESS TO FCεRI CROSSLINKING IN TERMS OF HISTAMINE AND CYTOKINE PRODUCTION: ROLE OF AUTOCRINE IL-3

R. Bricard, F. Machavoine, S. Mecheri, M. Begg, M. Leite de Moraes, E. Schneider, M. Dy

CNRS UMR 8147, Université Paris Descartes Paris, France; Institut Pasteur Paris France; GlaxoSmithKline Hertfordshire, UK

10.45 – 11.00
Oral presentation

MAST CELL BIOLOGICAL RESPONSES CAN BE AFFECTED BY IgE ALONE

A. Słodka, E. Bąbolewska, P. Witczak, E. Brzezińska-Błaszczyk

Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

11.00 – 11.30

Coffee break and poster viewing

11.30 – 13.25

HISTAMINE AND CELLS, part II
Chaired by: M. Ennis and D. Szukiewicz

11.30 – 12.15
Invited Lecture

MAREK JUTEL *(introduction by M. Ennis)*
THE ROLE OF HISTAMINE SIGNALLING IN PATHOMECHANISM OF NON-SPECIFIC IBD

Wroclaw Medical University, Department of Clinical Immunology, Wroclaw, Poland

12.15 – 12.30
Oral presentation

THE ANALYSIS OF MAST CELL AND EOSINOPHILIC INFILTRATES PRESENCE IN THE COURSE OF INFLAMMATORY BOWEL DISEASE

M. Pyzlak, J. Wejman, D. Jarosz, W. Tarnowski, G. Szewczyk, D. Szukiewicz

Department of General and Experimental Pathology, Medical University of Warsaw, Warsaw, Poland; Department of Pathology, W. Orlowski Clinical Hospital, Center for Medical Postgraduate Education, Warsaw, Poland; Department of Gastroenterology and Hepatology, Center for Medical Postgraduate Education, Warsaw, Poland; Department of Gastrointestinal Tract Surgery, W. Orlowski Clinical Hospital, Center for Medical Postgraduate Education, Warsaw, Poland

12.30 – 12.35
P21

HISTAMINE AND TOLL-LIKE RECEPTOR EXPRESSION ARE ALTERED IN PBMCs FROM INFLAMMATORY BOWEL DISEASE PATIENTS

S. Smolinska, P. Konieczna, M. Jutel, L. O'Mahony

Wroclaw Medical University, Department of Clinical Immunology, Wroclaw, Poland; Swiss Institute of Allergy and Asthma Research, Davos, Switzerland

12.35 – 12.40
P22

THE INFLUENCE OF HISTAMINE-RELATED GENES ON INFLAMMATION

T. Janikowski, M. Ciałoń, A. Jęda, G. Cwynar, J. Orchel, A. Witek, U. Mazurek

Department and Clinic of Gynecology and Obstetrics; Department of Molecular Biology, Medical University of Silesia, Katowice, Poland

12.40 – 12.55
Oral presentation

THE EXPRESSION OF HISTAMINE AND OTHER KEY MARKERS IN THE ZEBRAFISH GUT

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

Neuroscience Center and Institute of Biomedicine/Anatomy, University of Helsinki, Helsinki, Finland

12.55 – 13.10
Oral presentation

MAST CELLS GENERATE CYSTEINYL LEUKOTRIENES AND EXHIBIT ALTERED IgE-DEPENDENT RELEASABILITY UPON TLR3- AND TLR7-MEDIATED ACTIVATION
P. Witczak, A. Słodka, E. Bąbolewska, K. Wódz, E. Brzezińska-Błaszczyk
Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

13.10 - 13.15
P23

HOST DEFENSE PEPTIDE CATHELICIDIN LL-37 AS MAST CELL STIMULUS
E. Bąbolewska, A. Słodka, P. Witczak, A. Pietrzak, E. Brzezińska-Błaszczyk
Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland

13.15 – 13.20
P24

PRELIMINARY CHARACTERIZATION OF HISTAMINE RECEPTOR EXPRESSION IN HUMAN LUNG MAST CELLS
L.J. Kay, E.P. Seward, P.T. Peachell
University of Sheffield, Sheffield, UK

13.20 – 13.25
P25

EFFECT OF HISTAMINE ON HUMAN EPITHELIAL CELL LINES
J.B. Stott, P.L. Chazot, N. Lethbridge, A. Zholos, M. Ennis
Centre for Infection and Immunity, Queen's University Belfast, UK; School of Biological & Biomedical Sciences, Durham University, UK

13.25 – 13.30
P26

EFFECTIVENESS OF PHARMACOLOGICALLY INDUCED HISTAMINE RELEASE IN THE TREATMENT OF COMMON DISEASES IN DOGS
S. De La Torre, G. De Erausquin
De La Torre Veterinary Clinic, Mendoza, Argentina; University of South Florida, Tampa, USA

13.30 – 14.15

Lunch and poster viewing

14.15 – 14.35

DATABASES' PRESENTATION

14.15 – 14.25
Oral presentation

HISTAMINE H_4 LIGAND DATABASE
A. Sasse
School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland

14.25 – 14.35
Oral presentation

THE HISTAMINE METHODS & TOOLS DATABASE – READY TO USE
H.G. Schwelberger
Molecular Biology Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria

14.35 – 16.05

YOUNG INVESTIGATOR AWARD SYMPOSIUM
Chair and Jury: A. Sydbom, A. Sasse, J.-S. Lin

14.35 – 14.50
Oral presentation

DYNAMIC REGULATION OF WAKE-ACTIVE NEURONS
M. Sundvik, Y.-C. Chen, S. Rozov, P. Panula
Neuroscience Center and Institute of Biomedicine, Anatomy, Biomedicum Helsinki, University of Helsinki, Finland

14.50 – 15.05
Oral presentation

HISTAMINE MICROINJECTED INTO THE CEREBELLAR VERMIS IMPROVES MEMORY CONSOLIDATION OF INHIBITORY AVOIDANCE IN MICE
A.C. Gianlorenco, R. Mattioli
Federal University of Sao Carlos, Sao Carlos, Brazil

15.05 – 15.20
Oral presentation

EVIDENCE FOR ANALGESIC ACTION OF AESCULETIN IN CARRAGEENAN INDUCED INFLAMMATION

P. Rzodkiewicz, E. Gąsińska

Department of Biochemistry and Molecular Biology, Institute of Rheumatology, Warsaw, Poland

15.20 – 15.35
Oral presentation

EXPLORING TRANSCRIPTIONAL NETWORK CAUSALLY ASSOCIATED WITH POLLINOSIS BY TOLUENE-2,4-DIISOCYANATE-SENSITIZED RATS

M. Michioki, H. Mizuguchi, H. Ogishi, Y. Kitamura, N. Takeda, H. Fukui

Department of Molecular Pharmacology, Department of Otolaryngology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

15.35 – 15.50
Oral presentation

QUERCETIN INHIBITS TRANSCRIPTIONAL UP-REGULATION OF HISTAMINE H_1 RECEPTOR VIA SUPPRESSING PROTEIN KINASE C-D/EXTRACELLULAR SIGNAL-REGULATED KINASE/POLY(ADP-RIBOSE) POLYMERASE-1 SIGNALING PATHWAY IN HELA CELLS

T. Nakano, H. Mizuguchi, M. Hattori, Y. Baba, S. Ono, Q. Zhang, Y. Sasaki, M. Kobayashi, Y. Kitamura, N. Takeda, H. Fukui

Department of Molecular Pharmacology, Department of Otolaryngology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

15.50 – 16.05
Oral presentation

THE HUMAN HISTAMINEGIC SYSTEM IN HEALTH AND NEUROPSYCHIATRIC DISORDERS BRAIN: A POSTMORTEM STUDY

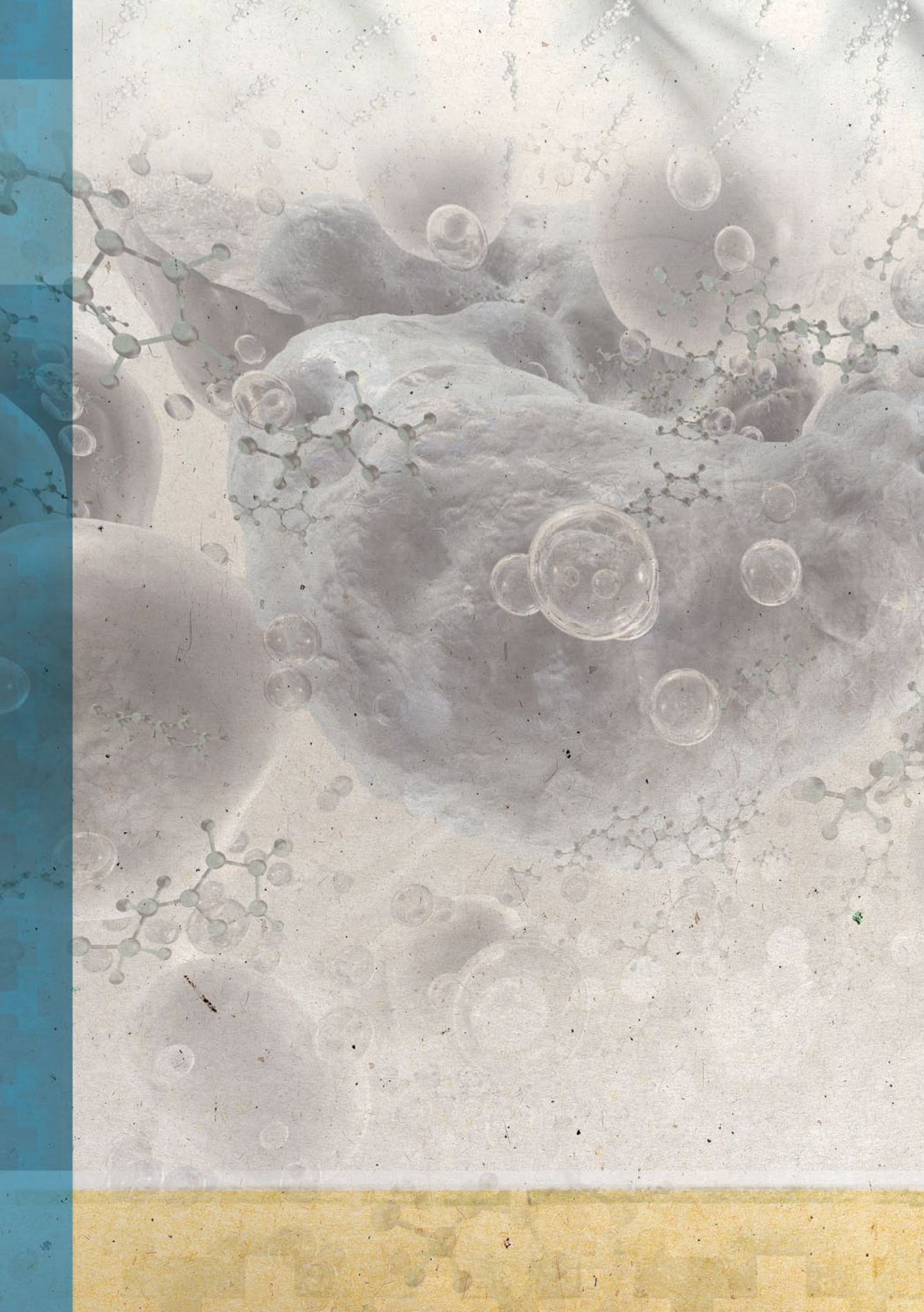
L. Shan, A.-M. Bao, D.F. Swaab

Netherlands Institute for Neuroscience, an Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands; Department of Neurobiology; Key Laboratory of Medical Neurobiology of Ministry of Health of China; Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou, China

16.05 – 16.30 — *Coffee break*

16.30 – 18.00 — **General Assembly of the EHRS**

19.30 – 00.00 — **Farewell Dinner**
Ambasador Centrum Hotel,
Restaurant



INVITED LECTURES

HISTAMINE AND ADDICTION: FROM BEHAVIOR TO NEUROTRANSMITTER INTERACTIONS

P. Panula, J. Vanhanen, T. Mäki, S. Rozov, S. Nuutinen

Neuroscience Center and Institute of Biomedicine, Anatomy, University of Helsinki, Finland; pertti.panula@helsinki.fi

Histamine has been implicated in mechanisms of reward, but which receptors and brain regions are involved have been poorly known. We found in 2001 that alcohol-preferring rats have high histamine and show reduced alcohol consumption following administration of histamine H_3 antagonists. To reveal the mechanisms in rodents, we utilized several mouse models. Several histamine H_3 receptor (H_3R) antagonists reduced alcohol-induced place preference and volunteer alcohol consumption in different mouse strains. H_3R KO mice showed lower alcohol consumption in the two-bottle choice test and drinking in the dark paradigm than control mice, and lower place preference. The effects on alcohol reward were dose-dependent. They were not observed in mice lacking histidine decarboxylase, suggesting that histamine is needed for the effect. We then developed a cue-induced reinstatement model of alcohol seeking using mice, and found that H_3R antagonists significantly reduced alcohol seeking.

Since drug reward is known to be dopamine-dependent, it is likely that the effects of H_3R antagonists indicate interactions with dopaminergic mechanisms. Thus, mechanistic studies to identify the relevant signalling pathways are needed. If H_3R antagonists are tested for use in human drug addicts, the strongly dose-dependent effects in rodents need to be considered.

HISTAMINE AT THE INTERSECTION OF CIRCADIAN RHYTHMS AND THE SLEEP-WAKE CYCLE?

W. Wisden, X. Yu, A.Y. Zecharia, Z. Ye, T. Goetz, S.G. Brickley, M.H. Hasting, N.P. Franks

Department of Life Sciences, Imperial College London, UK

The study of the sleep-wake cycle is usually guided by the flip-flop hypothesis. Wakefulness is promoted by tonic activity of "arousal neurons", which release amines such as histamine. Much interest centres on what turns these neurons off at the onset of sleep, keeps them off during sleep and then turns them on, or allows them to start firing again, shortly after waking [1]. According to the model, at the onset of sleep, and throughout sleep, the aminergic neurons are inhibited by GABAergic sleep-active neurons. Do GABAergic inputs onto histaminergic neurons in fact regulate sleep-wake behaviour? To address this we removed, genetically, ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors selectively from histidine decarboxylase (HDC)-expressing neurons. We recorded EEG profiles in non-tethered mice over 24 hours. Surprisingly, GABAergic transmission onto histaminergic neurons had no effect in regulating the natural sleep-wake cycle [2]. So perhaps other factors, additional to the GABA input, control the state-dependent firing of these arousal neurons during the sleep-wake cycle. We have been investigating how circadian clock genes, such as the transcription factor Bmal1, influence the activity of histaminergic neurons.

References

1. *Haas H, Panula P. Nat Rev Neurosci. 2003; 4:121-30.*
2. *Zecharia AY, Yu X, Götz T et al. J Neurosci. 2012; 32:13062-75.*

TOWARD SELECTIVE MOLECULAR TOOLS FOR HISTAMINE H_2 AND H_4 RECEPTORS: CONFORMATIONAL CONSTRAINTS, BIOISOSTERIC AND BIVALENT APPROACHES

A. Buschauer[1], R. Geyer[1], P. Igel[1], P. Baumeister[1], T. Birnkammer[1], D. Erdmann[1], U. Nordemann[1], N. Kagermeier[1], T. Holzammer[1], D. Wifling[1], J. Felixberger[1], D. Schnell[1], I. Brunskole[1], K. Ladova[1], K. Löffel[1], T. Reher[2], D. Neumann[2], Strasser[1], G. Bernhardt[1], R. Seifert[2], S. Dove[1]

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[2]*Institute of Pharmacology, Medical School of Hannover, 30625 Hannover, Germany*

Numerous compounds developed as subtype-selective ligands for histamine H_1 (H_1R) and H_2 receptors (H_2R) decades ago, have been proven to possess much higher affinity to H_3 (H_3R) and H_4 receptors (H_4R). This holds, e.g., for imidazole-type ligands such as the potent H_4R agonist 5-methylhistamine, which was initially described as the first selective H_2R agonist, as well as for guanidines derived from impromidine.

Aiming at subtype-selective, radiolabeled and fluorescent H_xR ligands as pharmacological tools, bioisosteric and bivalent approaches were explored in our laboratory, starting from guanidine-type H_2R agonists or piperidinomethyl-phenoxypropylamine-type H_2R antagonists, respectively. Modification of the latter gave tritiated ([^{3}H]UR-DE257) and fluorescent ligands for the H_2R and paved the way to fluorescent H_3R antagonists. In the guanidine series, the replacement of the imidazole ring by 2-aminothiazole in combination with an acyl- or carbamoylguanidine moiety resulted in highly potent and selective H_2R agonists, including bivalent agonists. With regard to H_4R selectivity, the suitability of guanidine replacements, various heterocycles and conformationally constrained linkers was explored, and the substitution pattern of acylguanidines and cyanoguanidines was varied, resulting, e. g., in the high-affinity $H_{3/4}R$ radioligand [^{3}H]UR-PI294 and the potent H_4R agonists UR-PI376 and trans-(+)-(S,S)-UR-RG98.

Selectivity for H_4R over H_3R is especially challenging. Beyond HR subtype selectivity, activities of many ligands differ significantly from those at the human H_4R, especially at rodent H_4Rs. Such differences were extremely pronounced in case of proximal readouts ([^{32}P]GTPase, [^{35}S]GTPγS assay). Thus, with respect to predictivity, orthologue- and assay-dependent activity profiles should be considered (e.g., human, murine and rat H_4Rs including mutants (binding and functional data, GPTase, GTPγS assay), reporter gene and arrestin assays, native cells).

HISTAMINE SYNTHESIS AND ITS FUNCTIONS IN MURINE MAST CELLS

S. Tanaka

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Histamine regulates a wide variety of pathological and physiological responses, such as inflammation, gastric acid secretion, neurotransmission, and immune modulation, which are mediated by its specific membrane receptors, H_1, H_2, H_3, and H_4 subtypes. An array of studies have revealed how these receptors regulates histamine-mediated responses, although it remains insufficiently understood how histamine synthesis is regulated and when histamine plays a dominant role in these responses. We have focused on the study associated with histamine synthesis, which is catalyzed by histidine decarboxylase (HDC). Over a decade ago, the $Hdc^{-/-}$ mice were generated, and histamine synthesis was found to be abolished in these mice. Mast cells in the $Hdc^{-/-}$ mice exhibited aberrant morphology with severely decreased granule contents, although the roles of histamine in granule maturation remained unknown. Here, we investigated the roles of histamine in granule maturation of murine mast cells using the $Hdc^{-/-}$ mice and our culture model of cutaneous mast cells. The absence of HDC had a major impact on mature mast cells but not on IL-3-dependent bone marrow-derived cultured mast cells. Our findings strongly suggest that histamine should promote granule maturation of murine mast cells in an autocrine fashion and a large part of these functions might be independent of the known histamine receptors. The immunological mediators and bioactive compounds that promote mast cell growth and differentiation, such as IL-3, stem cell factor, IgE, and butyrate, all have a potential to induce a transient increase in histamine synthesis. Local inhibition of histamine synthesis might be a novel therapeutic approach for chronic allergy and inflammatory diseases through suppression of granule maturation of accumulating mast cells.

HISTAMINE SIGNALING IN THE PATHOMECHANISMS OF INFLAMMATORY BOWEL DISEASE (IBD)

M. Jutel

Department of Clinical Immunology, Wroclaw Medical University, Chalubinskiego 5, 50-368 Wrocław, Poland

The role of histamine signal in the pathogenesis inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and chronic nonspecific ulcerative colitis (UC) has been investigated. In particular the molecular basis and the possible therapeutic use of the specific H_1R, H_2R and H_4R signals deserves attention considering their potential in the modulating of the chronic inflammatory process in the gut. It has been well established that differential expression of various histamine receptor types in immune competent cells may determine either the enhancement of suppression of the inflammatory effector mechanisms in the tissues such as the gut. In addition, the modulation of the production of endogenous histamine plays an important role in the pathomechanisms of IBD.

It has been demonstrated that IBD patients show impaired synthesis of histamine in immune competent cells. Moreover, these cells, especially CD4+ T cells demonstrate marked differences in histamine receptor expression patterns as compared to healthy controls. This results in different potential of histamine to suppress toll-like receptor-induced inflammatory effector mechanisms responsible for initiation and progression of IBD.

The interplay and reciprocal regulation of histamine and TLR signaling might be of great importance for the development of new therapeutic strategies for IBD.

COMMUNICATIONS

HISTAMINERGIC NEURONS ARE EXITED BY PROTONS

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Extracellular levels of CO_2 vary in response to alterations of blood oxygen levels and are fundamental physicochemical signals controlling breathing and wakefulness. Many of the histaminergic neurons in the tuberomamillary nucleus (TMN), in particular their dendrites where the firing is generated, lie very close to the brain surface, the median eminence, where they are likely sensing the composition of the cerebrospinal fluid. They have been shown to be involved in CO_2-mediated arousal using cFos histochemistry. We therefore investigated the mechanisms of histaminergic neurons responses to changes in extracellular levels of acid/CO_2. Recordings in rat brain slices revealed that acidification within the physiological range (pH from 7.4 to 7.0) excites histaminergic neurons. This excitation is significantly reduced by mGluR I antagonists and abolished by i) benzamil, an antagonist of acid sensing ion channels (ASICs), ii) Na^+/Ca^{2+} exchanger and iii) ouabain which blocks Na^+/K^+ ATPase. We found variable combinations of four known types of ASICs in single TMN neurons, and observed activation of ASICs in single dissociated TMN neurons only at a pH below 7.0. Glutamate, which can be released by glial cells and hypocretin/orexin neurons, enhances the acid/CO_2-induced activation of TMN neurons. This amplification demands the coordinated function of metabotropic glutamate receptors, Na^+/Ca^{2+} exchanger and Na^+/K^+ ATPase. The participation of TRPV1-channels is under investigation. These results are relevant for understanding the neuronal mechanisms controlling acid/CO_2-induced arousal e.g. in obstructive sleep apnoea.

ABT-239, AN H_3 RECEPTOR ANTAGONIST / INVERSE AGONIST, INCREASES HISTAMINE RELEASE AND C-FOS EXPRESSION IN BRAIN REGIONS INVOLVED IN MEMORY AND COGNITION

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Pharmacological blockade of H_3Rs exerts procognitive effects, increase wakefulness and reduce bodyweight in animal models. ABT-239 is a selective, non-imidazole H_3R antagonist/inverse agonist that improved acquisition of a five-trial, inhibitory avoidance test in rat pups, and social memory in adult and aged rats. The aim of this work was to find out if ABT-239 modulates the release of histamine and the expression of c-Fos, a marker of neuronal activation, in brain areas crucial for memory processes which receive histaminergic innervation, such as the prefrontal cortex (pFCx) and nucleus basalis magnocellularis (NBM). We perform a dual-probe microdialysis study, where one probe was inserted in the TMN and another one, in a histaminergic projection area: pFCx, nucleus accumbens (NAcc), NBM and striatum (STR). The perfusion of the TMN with ABT-239 (10 μM) for 60 minutes increased histamine release from the TMN, NBM and pFCx, but not from the STR or NAcc. Using immunohistochemistry, we observed that the same treatment determines a significant increase of the c-Fos expression, a known marker of neuronal activation, in the NBM and pFCx, but not in the STR or Nacc. Interestingly, intracerebroventricular pretreatment with α-fluoromethylhistidine (α-FMH), a histamine synthesis inhibitor, prevented the increase of c-Fos expression in the pFCx and NBM after TMN perfusion with ABT-239. This observation fits well with the report that ABT239 lacks procognitive effects in histamine deprived mice (HDC-KO and α-FMH-treated mice). Thus, defined by their sensitivity to ABT-239, histaminergic neurons establish distinct pathways according to their terminal projections, and modulate neurotransmitter release and neuronal activation in regions crucial for memory processes.

BEHAVIOURAL EVIDENCE FOR CENTRAL H_4 HISTAMINE RECEPTORS

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We provided the first evidence for functional H_4 histamine receptors on 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 H_4 receptor. In this present study, we investigated whether JNJ7777120, a selective centrally-active H_4R antagonist has any behavioural effects on BALB/c mice, using our novel elevated platform open space anxiety test, and an open field object recognition test (NOR). Four groups of 3 month BALB/c mice (n = 8) were injected with saline, 5, 10 and 20 mg/kg i.p, JNJ7777120, respectively, 30 minutes prior to exposure to an elevated platform for a single 12 minute session as described in [2]. The same mice were then exposed to an open field with a single object positioned centrally, for two daily 12 minute sessions. Then the mice were injected with saline, 5, 10 and 20 mg/kg i.p. JNJ7777120 30 minutes prior to exposure to two identical objects (sample phase, 6 minutes). After 24 hrs delay, mice were re-exposed to an identical copy of an object shown before, now familiar, alongside a novel object (choice phase, 3 minutes). In contrast to diazepam, neither saline nor JNJ7777120 at all doses tested, prompted any entries onto slopes. 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. In the NOR test, no improvement in memory performance was detected, but a dose-dependent increase in approaches to objects was observed with JNJ7777120. Overall, these results suggest that antagonizing the H_4R has no anxiolytic or novel object recognition memory enhancing effects in BALB/c mice.

References

1. *Connelly WM, Shenton FC, Lethbridge N et al. Br J Pharmacol. 2009; 157: 55-63.*
2. *Ennaceur A, Michalikova S, van Rensburg R, Chazot PL. Behav Brain Res. 2010; 207: 84-98.*

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THE HISTAMINE H_4 RECEPTOR IS FUNCTIONALLY EXPRESSED ON RAT SUBSTANCE P-CONTAINING DORSAL ROOT GANGLIA

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We have previously reported evidence (using double labelling approaches) that histamine H_4Rs are expressed on C-fibres (substance P +ve cells) at the level of the skin and DRGs [1], which is contrary to the H_3 receptor, which we have previously shown to be present only on subsets of Aδ fibres [2].

A model system is required to study the functional and pharmacological properties of neuronal H_4R. Using isolated primary rat DRG neurons, histamine and VUF8430 (selective H_4R agonist) were found to stimulate an intracellular [Ca^{2+}] influx. 1 μM of VUF8430 produced an influx of free intracellular calcium in a subset of cultured primary rat DRG neurons. These neurons were thought to be skin-specific due to their apparent capsaicin sensitivity (VUF8430, mean EC_{50}= 4.75 x 10^{-8}M). This signal was suppressed by 1 μM JNJ7777120 (selective H_4R antagonist). This is in line with recent findings, where histamine-induced free [Ca^{2+}] influx is related to capsaicin sensitivity (histamine-induced free [Ca^{2+}] influx in DRG neurones from wild type mice but not TRPV1 -/- mice) [3]. Interestingly, we have pilot data which suggests that the H_4R is functional expressed on DRG associated glial cells.

References

1. *Katebe MA, Chazot PL. Inflamm Res (Suppl 2) 61, S60.*
2. *Cannon KE, Chazot PL, Hann V, Shenton F, Hough LB, Rice FL. Pain. 2007; 129: 76-92.*
3. *Hsieh GC, Chandran P, Salyers AK et al. Pharmacol Biochem Behav. 2010; 95: 41-50.*

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HISTAMINE TRANSPORT BY MOUSE PLASMA MEMBRANE MONOAMINE TRANSPORTER AND MOUSE ORGANIC CATION TRANSPORTER 3

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Histamine clearance is an essential process for avoiding excessive histaminergic neuronal activities. Although the molecular mechanism of histamine clearance had remained largely unclear, we recently revealed that plasma membrane monoamine transporter (PMAT) and organic cation transporter 3 (OCT3) played the pivotal role in histamine clearance by primary human astrocytes. These transporters belong to the low-affinity and high-capacity transporters, and the K_m values of human PMAT and OCT3 to histamine are 4.4 mM and 0.6 mM, respectively. However, the involvement of these transporters in *in vivo* histamine clearance has remained unclear.

Before we investigated the function of the two transporters expressed in mouse brain, we confirmed the transport ability of mouse Pmat (mPmat) or mOct3 using 293T cells overexpressing mPmat or mOct3. The transport assay revealed that K_m and V_{max} values of mPmat to histamine were 6.4 ± 1.2 mM and 8.2 ± 0.79 nmol/mg protein/min and those of mOct3 were 2.5 ± 0.56 mM and 3.1 ± 2.3 nmol/mg protein/min. Next, we directly measured the extracellular concentration of histamine in the hypothalamus using *in vivo* microdialysis method after injection of decynium-22 (d22), a common inhibitor of mPmat and mOct3. The d22 injection increased histamine concentration in a dose-dependent manner, suggesting that mPmat and/or mOct3 were actually involved in histamine clearance in mouse brain.

In the present study, we showed the importance of mPmat and mOct3 in histamine clearance. This study might lead to a better understanding of the histaminergic nervous system.

PHENYTOIN DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS IN EPILEPSY MODELS IN RATS

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The standard antiepileptic drug Phenytoin (PHT) is in use for epilepsy since 1937, however, patients experience unwanted side effects during its use, e.g. sedation, attention deficit, and disturbance of memory. Mounting experimental and clinical findings support the inference of H_3 receptor (H_3R) antagonists on convulsions in different seizure models in addition to cognition and sleep behaviour. Therefore, a new class of piperidine-substituted PHT derivatives has been developed and investigated on their anticonvulsive effects in two epilepsy models. ST-1394 and ST-1395 are parent ligands for this new class as hybride molecules of the robust H_3Rs pharmacophore 3-piperidinopropyloxy moiety in combination with the hydantoin element of PHT. Here, in addition to *in vitro* binding studies at hH_3R, the protections of ST-1394 and ST-1395 against MES-induced and PTZ-kindled seizures in rats have been studied as model on epilepsy.

ST-1394 and ST-1395 showed affinities at hH_3R in the nanomolar concentration range (pK_i values of 6.2 and 7.1, respectively). In MES model PHT (10 mg/kg i.p.) fully protected animals, whereas animals pre-treated with 1 mg/kg, i.p. of ST-1394 were moderately protected. Contrary, ST-1394 (1 mg/kg, i.p.) failed to modify PTZ-kindled convulsion. However, a dose of 10 mg/kg significantly reduced convulsions in both seizure models. Surprisingly, ST-1395 (1, 5, and 10 mg/kg, i.p.) showed epileptogenic effect in MES model, and these results were confirmed in PTZ model, as no protection was observed against seizure in doses tested (1 and 10 mg/kg). Our results increase the evidence of potential protection for H_3R ligands in convulsions and may offer further perspectives in anti-epileptic pharmacotherapy. New derivatives and epilepsy models are necessary to elucidate the discrepant results and to obtain multi-targeting ligands combining pro-cognitive property of H_3R antagonists with anticonvulsant effect.

P2

THE HYPOPHAGIC FACTOR OLEOYLETHANOLAMIDE RECRUITES HYPOTHALAMIC HISTAMINE PATHWAYS TO INHIBIT FOOD INTAKE

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Feeding behaviour is integrated primarily through hypothalamic neuronal networks. Brain histamine exerts its anorectic effect by activating hypothalamic paraventricular (PVN) and ventromedial nuclei (VMH). Oleoylethanolamide (OEA) is a lipid-amide released by intestinal cells in response to fat intake and indirectly conveys satiety signaling to hypothalamic nuclei. Systemic administration of OEA activates oxytocin (OT) neurons in the PVN/supraoptic nucleus (SON), and OT appears to mediate OEA anorectic effect [1]. We showed that OEA requires the integrity of histamine neurotransmission to fully display its hypophagic effect and that OEA-induced c-Fos expression in the PVN is attenuated in HDC-KO mice [2]. To test if OEA signaling recruits histamine neurons to activate OT neurons in the PVN, we investigated the effect of histamine-deficiency on OEA-induced c-Fos expression in OT neurons by immunofluorescent histochemistry. Wild type (WT, n=4) or histidine decarboxylase (HDC-KO; n=4) mice were fasted for 12h, then treated with OEA (10 mg/kg, i.p.) and sacrificed after 2 hours. OEA administration increased c-Fos expression in the PVN/SON of WT mice only ($P<0.01$), not in the HDC-KO mice. The ratio of c-Fos/OT co-expressing cells in the PVN in the HDC-KO mice was significantly lower than that in the WT mice after OEA injection ($P<0.01$). As the regulating system of feeding behavior involves several hypothalamic nuclei that receive histaminergic projections, we also investigated OEA-induced c-Fos expression in other feeding-related hypothalamic areas in WT and HDC-KO mice. In the VMH, no significant induction of c-Fos was observed after OEA injection in either genotypes. In the arcuate nucleus (ARC), c-Fos positive cells were detected in both OEA-treated genotypes, with no significant differences. Our results suggest that OEA involves neuronal histamine to activate the OT neurons in the PVN specifically to induce hypophagia.

References

1. *Gaetani S, Fu J, Cassano T, Dipasquale P et al., J Neurosci. 2010; 30: 8096-101.*
2. *Provensi et al., EHRS 2011.*

CARDIOVASCULAR EFFECTS OF HISTAMINE H_3 RECEPTOR INVERSE AGONISTS IN HAEMORRHAGE-SHOCKED RATS

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In the cardiovascular system, presynaptic histamine H_3 receptors play the role of heteroreceptors which regulate the secretion of other neurotransmitters (e.g. noradrenalin from postganglionic sympathetic nerve fibres which innervate the heart and blood vessels). Administration of H_3 receptor inverse agonists may lead to an increase in noradrenalin release, whereas their agonists evoke an opposite effect. Integrated response to blood loss/haemorrhagic shock consists of two phases: an initial sympathoexcitatory phase initiated from arterial baroreceptors, and a second, hypotensive phase, characterized by a generalized sympathoinhibition. The aim of the present study was to examine haemodynamic effects of two H_3 receptor inverse agonists on cardiovascular regulation in the sympathoinhibitory phase of cardiovascular regulation in haemorrhagic shock. Experiments were carried out in ketamine/xylazine-anaesthetised male Wistar rats subjected to haemorrhagic hypotension, with mean arterial pressure (MAP) stabilized at 20-25 mmHg, which resulted in the death of all control animals within 30 min. Both clobenpropit (2 and 5 μmol/kg) and A-331440 (2 μmol/kg) given intravenously (iv) at 5 min of critical hypotension evoked long-lasting increases in MAP, pulse pressure and regional peripheral blood flows, with an improvement in survival at 2 h. The effect was inhibited by chemical sympathectomy with 6-hydroxydopamine (50 mg/kg, subcutaneously for 3 days) and pre-treatment with α1-adrenoceptor antagonist prazosin (0.5 mg/kg, iv) but not with β-adrenoceptor blocker propranolol (1 mg/kg, iv). In conclusion, the results of the present study demonstrate resuscitating effects of H_3 receptor inverse agonists in haemorrhagic shock in rats. The involvement of noradrenergic postganglionic sympathetic nerve fibres can be suggested.

ENHANCED HISTAMINERGIC NEUROTRANSMISSION AND SLEEP-WAKE ALTERATIONS, A STUDY IN HISTAMINE H_3-RECEPTOR KNOCK-OUT MICE

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Long-term abolition of a brain arousal system impairs wakefulness (W), but little is known about the consequences of long-term enhancement. The histaminergic arousal system is under the negative control of H_3-autoreceptors whose deletion results in permanent enhancement of histamine (HA) turnover. In order to determine the consequences of enhancement of the histaminergic system, we compared the cortical EEG and sleep-wake states of H_3-receptor knockout (H_3R-/-) and wild-type mouse littermates. We found that H_3R-/-mice had rich phenotypes. On the one hand, they showed clear signs of enhanced HA neurotransmission and vigilance, i.e., a higher EEG θ power during spontaneous W and a greater extent of W or sleep restriction during behavioral tasks, including environmental change, locomotion, and motivation tests. On the other hand, during the baseline dark period, they displayed deficient W and signs of sleep deterioration, such as pronounced sleep fragmentation and reduced cortical slow activity during slow wave sleep (SWS), most likely due to a desensitization of postsynaptic histaminergic receptors as a result of constant HA release. Ciproxifan (H_3-receptor inverse agonist) enhanced W in wild-type mice, but not in H_3R-/-mice, indicating a functional deletion of H_3-receptors, whereas triprolidine (postsynaptic H_1-receptor antagonist) or α-fluoromethylhistidine (HA-synthesis inhibitor) caused a greater SWS increase in H_3R-/- than in wild-type mice, consistent with enhanced HA neurotransmission. These sleep-wake characteristics and the obesity phenotypes previously reported in this animal model suggest that chronic enhancement of histaminergic neurotransmission eventually compromises the arousal system, leading to sleep-wake, behavioral, and metabolic disorders similar to those caused by voluntary sleep restriction in humans.

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HISTAMINERGIC TUBEROMAMILLARY NUCLEUS CONSTITUTES ONE OF THE MOST IMPORTANT TARGETS FOR THE WAKE-PROMOTING EFFECT OF OREXIN NEURONS BUT NOT THE EXCLUSIVE ONE

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Histamine (HA) and orexin (Ox) neurons constitute two major hypothalamic wake-promoting systems. Given the dense excitatory Ox inputs to HArgic tuberomamillary neurons it has been suggested that the arousing role of Ox depends on the HA transmission. To test this hypothesis, we performed neuroanatomical study on the Ox system in mice and cats and studied the effects of Ox ligands on the sleep-wake cycle of wild-type (WT) and histidine decarboxylase knockout (HDC-/-) mice. Cat and mouse brain sections were subjected to immunohistochemical identification of Ox fibers/terminal-like dots in the histaminergic, cholinergic, dopaminergic and noradrenergic nuclei. Mice were chronically implanted with electrodes for cortical EEG and sleep-wake monitoring under baseline conditions (12h light/dark cycle) and pharmacological dosing. Ox-A or B (1, 3 & 10 µg) was injected by intracerebroventricular route whereas SB-334867 (15 mg/kg), an Ox1-receptor antagonist, by intraperitoneal one. We found in both cats and mice a particularly dense innervation of Ox fibers/terminal-like dots on the tuberomammillary nucleus, cholinergic mesopontine tegmentum (++++), noradrenergic locus coeruleus (+++); a less dense innervation on the cholinergic basal forebrain (++) and a scattered innervation on the dopaminergic substantia nigra (+). We also found that both Ox-A and B produced prompt and strong arousal and dose-dependently enhanced the duration of active wakefulness in WT and HDC-/-mice. The effects were identical in the two genotypes and no statistical difference in any of the doses used was found. Similarly, SB334867 enhanced slow wave sleep and decreased waking and paradoxical sleep. The effects were identical in the both genotypes. Thus, Ox neurons can activate brain targets other than HA neurons to generate brain arousal. The HArgic tuberomamillary nucleus constitutes one of the most important targets for the wake-promoting effect of Ox neurons but not the exclusive one.

HISTAMINE H_3 RECEPTOR ACTIVITY OF (HOMO)PIPERIDINYL-PENTOXYPHENYL DERIVATIVES

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Histamine H_3 receptor regulates release of histamine itself and other neurotransmitters such as acetylcholine, dopamine, norepinephrine, glutamate and serotonin. Histamine H_3 receptor antagonists/inverse agonists could be used in the treatment of cognitive (ADHD, Alzheimer's disease, schizophrenia) and sleep disorders (narcolepsy) as well as obesity and neuropathic pain [1, 2]. Many pharmaceutical companies and academic research groups have synthesized a large variety of highly potent histamine H_3 receptor ligands (for review see e.g. [3, 4])

The aim of this study was the extension of our previous work in the search for non-imidazole histamine H_3 receptor ligands [5,6]. Two series of pentoxyphenyl derivatives having piperidine and homopiperidine moieties were prepared according to known methods [6]. Compounds were screened for their binding affinities at recombinant human histamine H_3 receptor and exhibited pronounced to high affinities (K_i values from 9 to 427 nM).

Affinity at human histamine H_3R was not directly connected with the kind of sec. amine moiety (piperidine or homopiperidine) but straightly depended on substituent in a phenyl ring. More voluminous substituents, e.g. biphenyl, tert-amyl, were better tolerated maintaining affinity than smaller ones e.g. chloro substituents or methyl groups.

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References
1. *Tiligada E, Kyriakidis K, Chazot PL, Passani MB. CNS Neurosci Ther. 2011; 17: 620-8.*
2. *Tiligada E, Zampeli E, Sander K, Stark H. Expert Opin Investig Drugs. 2009; 18: 1519-31.*
3. *Sander K, Kottke T, Stark H. Biol Pharm Bull. 2008; 31: 2163-81.*
4. *Łazewska D, Kieć-Kononowicz K. Expert Opin Ther Pat. 2010; 20: 1147-69.*
5. *Łazewska D, Ligneau X, Schwartz JC, Schunack W, Stark H, Kieć-Kononowicz K. Bioorg Med Chem. 2006; 14: 3522-9.*
6. *Łazewska D, Kuder K, Ligneau X et al. Bioorg Med Chem. 2009; 17: 3037-42.*

NON-IMIDAZOLE HISTAMINE H_3 LIGANDS. SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL INVESTIGATION OF 1-[2-THIAZOL-5-YL- AND 1-[2-THIAZOL-4-YL-(2-AMINOETHYL)]-4-N-PROPYLPIPERAZINE DERIVATIVES

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Early generation of H_3 receptor ligands were based on structures containing the imidazole moiety, many of which have found utility as pharmacological tools However, antagonist carrying on the imidazole heterocycle is the potential issue for drug-drug interactions through inhibition of hepatic cytochrome P_{450} enzymes and poor CNS penetration. For these reasons efforts have been directed toward the discovery of H_3 antagonists without an imidazole moiety as these compounds may offer improvements in binding affinity, CNS penetration, and reduced potential for cytochrome P_{450} enzymes inhibition.

Previously, our laboratory has described several non-imidazole piperazine-based histamine H_3 antagonists, consisting of 1-(2-thiazolobenzo)- and, 1-(2-thiazolopyridine)- moieties. In continuation of our earlier work, we studied the influence, on H_3-receptor antagonistic activity, of the introduction of 2-CH_3-2-R-aminoethyl- substitution at position 5 and 4 of the thiazole ring, respectively. Therefore, the series of 1-[2-thiazol-5-yl)- and 1-[2-thiazol-5-yl-(2-aminoethyl)]-4-n-propylpiperazines were prepared and pharmacological evaluated (electric field stimulation assay on guinea-pig jejunum). We observed, that the position 5 of 2-methyl-2-R-aminoethyl-substituents in the thiazole ring is favourable for histamine H_3 receptor antagonist activity, whereas its presence in position 4 leads, in almost all cases, to strong decrease of the activity.

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P11

BENZYLPIPERIDINE VARIATIONS ON HISTAMINE H_3 RECEPTOR ANTAGONISTS FOR IMPROVED DRUGLIKENESS

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Within the recent years several novel histamine H_3 receptor (H_3R) antagonists/ inverse agonists have entered clinical phases addressing a broad therapeutic variety of mainly centrally occurring diseases [1]. Nevertheless, many promising (preclinical) candidates failed due to their pharmacokinetic profile, mostly because of high lipophilicities and of dibasic characters [2]. Analysis of previously as potential PET ligands synthesized compounds in the series of benzylpiperidines (ST-889, ST-928) revealed promising results concerning physicochemical properties and druglikeness [3].
The design, the synthesis, the evaluation of the binding properties at hH3R and the calculated estimation of different physicochemical and druglikeness properties of further benzylpiperidine variations on H_3R antagonists will be shown. Due to the introduction of various small hydrophilic parts in the structure, drug likeness parameters have been improved. Compounds ST-1032 and ST-1073 showed in addition to high affinity at the H_3R pKi values (hH3R) of 9.3 and 8.6, and clogP values of 2.2 and 3.4, and LELP values of 6.8 and 7.4 have also been calculated, respectively.
The data clearly show the high potential for further optimization on pharmacokinetic and druglike properties in this series of H_3R antagonists.

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References

1. *Tiligada E, Kyriakidis K, Chazot PL, Passani MB. CNS Neurosci Ther. 2011; 17: 620-8.*
2. *Walter M, Stark H. Front Biosci (Schol Ed). 2012; 4: 461-88.*
3. *Isensee K, Amon M, Garlapati A et al. Bioorg. Med. Chem. Lett. Bioorg Med Chem Lett. 2009; 19: 2172-5.*

AMIDE DERIVATIVES OF 4-METHYLPIPERAZIE AS HISTAMINE H_4 RECEPTOR LIGANDS

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The histamine H_4 receptor (H_4R) is widely expressed in cells involved in immune response and inflammation [1]. Anti-H_4R receptor ligands were evaluated in animals models of some diseases (e.g. allergic rhinitis, airway inflammation, pruritus, itch or pain) and showed positive effects [2]. In 2003 Jablonowski *et al.* published a series of selective H_4R antagonists with JNJ7777120 the first orally potent and selective non-imidazole H_4R compound [3]. Since that time many potent and selective H_4R ligands (antagonists/inverse agonists) have been synthesized [4,5].
Our research group is also involved in the search for histamine H_4R ligands. The aim of this study was the continuation of our previous work in the search for H_4R active structures among amide analogues of JNJ7777120 [6]. Two series of compounds with 4-methylpiperazine motif were prepared, in one 4-methylpiperazine moiety was directly connected *via* amide group with a substituent and in the other one a propyl linker between 4-methylpiperazine moiety and amide group has been introduced. All compounds were screened for their binding affinities at recombinant human H_4R expressed in SF9 cells and co-expressed with G-protein $G\alpha_{i2}$ and $G\beta_{1\gamma2}$ subunits. Affinities of all synthesized compounds are in micromolar concentration range (K_i values ≥ 1 μM). Generally compounds with the alkyl linker displayed lower affinities than their directly connected with 4-methylpiperazine ring analogues.

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References
1. *Zhang M, Thurmond RL, Dunford PJ. Pharmacol Ther. 2007; 113: 594-606.*
2. *Tiligada E, Zampeli E, Sander K, Stark H. Expert Opin Investig Drugs. 2009; 18: 1519-31.*
3. *Jablonowski JA, Grice CA, Chai W et al. J Med Chem. 2003; 46: 3957-60.*
4. *Łażewska D., Kieć-Kononowicz K. Front Biosci (Schol Ed). 2012; 4: 967-87.*
5. *Engelhardt H, Smits RA, Leurs R, Haaksma E, de Esch IJ. Curr Opin Drug Discov Devel. 2009; 12: 628-43.*
6. *Karcz T. et al. Inflamm Res. 2010, 59, S243.*

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SEARCH FOR NEW HISTAMINE H_4 RECEPTOR LIGANDS IN THE GROUP OF 1,3,5-TRIAZINE DERIVATIVES

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Currently it is known that histamine fulfils its effects through four histamine receptor subtypes (H_1-H_4). They all belong to the GPCRs family, and they are different in both functions and distribution in the body. The subject of our interest is the histamine H_4 receptor, which was discovered and cloned by several independent research teams in 2000-2001 [1]. It is located primarily in immune system cells, suggesting its participation in the body's immune responses, allergic and inflammatory conditions. The positive effect of H_4 antagonists was observed in vivo in asthma, hay fever, pruritus or bowel inflammation [2,3]. The first orally active, potent and selective H_4 receptor antagonist, was non-imidazole derivative - JNJ 7777120, described in 2003 [4].

Up to now, there are many well-known compounds which are selectively interacting with histamine H_4 receptor [5]. Among them there is a large group of mono- , di- and triazine derivatives [6]. For this reason our work is focused on the search for new histamine H_4 receptor ligands in the group of 1,3,5-triazine derivatives. Presented research results involve newly obtained 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives possessing in 6-position different phenoxymethyl substituents.

As the result of our studies 12 new compounds were obtained by the direct reaction of appropriate carboxylic esters with guanidine derivative. Subsequently they were evaluated for their affinity at human H_4R with radioligand binding assays using [^{3}H]histamine as radioligand. In silico predictions of toxicity and drug-likeness by newly obtained compounds were also carried out.

The affinity of newly obtained compounds has shown noticeable susceptibility to the pattern of 6-position substituent modifications. The most potent compound showed K_i value of ca. 6 μM.

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References

1. *Nguyen T, Shapiro DA, George SR et al. Mol Pharmacol. 2001; 59: 427-33.*
2. *Walter M, Kottke T, Stark H. Eur J Pharmacol. 2011; 668: 1-5.*
3. *Tiligada E, Zampeli E, Sander K, Stark H. Expert Opin Investig Drugs. 2009; 18: 1519-31.*
4. *Jablonowski JA, Grice CA, Chai W et al. J Med Chem. 2003; 46: 3957-60.*
5. *Kiss R, Keserű GM. Expert Opin Ther Pat. 2012; 22: 205-21.*
6. *Łażewska D, Kieć-Kononowicz K. Front Biosci (Schol Ed). 2012; 4: 967-87.*

IN VITRO CYTOTOXICITY EVALUATION OF 3 PIPERIDINOPROPAN-1-OL DERIVATIVES WITH H_3R ACTIVITY AND 1,3,5-TRIAZINE DERIVATIVES WITH H4R ACTIVITY

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Cytotoxicity assays are widely used in drug discovery research for testing the safety of therapeutic agents before they are progressed to the clinic. On the other hand, cytotoxicity assays are an useful tools in research of new anticancer agents. Additionally, in vitro toxicity testing is a reliable alternative to toxicity testing by reduction, replacement and refinement of animals (3Rs approach).

In the present study, 3 piperidinopropan-1-ol derivatives: DL-76, DL-77 and 1,3,5-triazine derivatives: KB-2, KB-4, KB-20, KB-35T, JN-25.1 were screened for cytotoxicity against HEK-293 (Human Embryonic Kidney cell line) and IMR-32 (Human Neuroblastoma cell line) with use of short term colorimetric EZ4U cell proliferation and cytotoxicity assay protocol. The compounds were examined for cytotoxic activity under various concentrations and compared to the doxorubicine as a standard drug.

Data indicates that among all compounds KB-2, KB-4 displayed the highest cytotoxic activity against HEK-293 and IMR-32 after 48 h. Additionally similar activity was observed for JN-25.1 but only against IMR-32 cell line. However, the significant effects were observed under more then 10-fold higher concentrations (against HEK-293) and more than 1000-fold (against IMR-32) in compare to doxorubicine.

The obtained results showed, that all compounds were found to be devoid of any significant activity against IMR-32 cell line and KB-2 and KB-4 possess weak cytotoxic activity against HEK-293 cell line.

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PIPERAZINE MODIFICATION IN 2,4,6-TRIAMINOPYRIMIDINE DERIVATIVES AS HISTAMINE H_4 RECEPTOR LIGANDS

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The human histamine H_4 receptor (hH_4R) is a promising new target in the therapy of inflammatory and immunomodulatory diseases. The 2,4,6-triaminopyrimidine structure has been established as a potent hH_4R pharmacophore.

Starting with the inverse agonist ST-1012 as reference ligand, piperazine modifications were performed to get larger variations in amine linkage. Different spacers were introduced into the lead structure and the influence of this linker on affinity was evaluated by measurement of hH_4R affinity on membranes of a transient expression system with co-expression of G proteins. Nine new 2,4,6-triaminopyrimidines were synthesized . Furthermore, the ligand efficiency was calculated for each compound to verify the druglikeness and evaluate potential new lead structures. All new compounds showed affinities at hH_4R in the micromolar concentration range and promising ligand efficiencies. While a short distance between aminopyrimidine and basic moiety is beneficial, a lipophilic group in the eastern part is necessary to maintain hH_4R affinity.

This work has kindly been promoted by the Hessian LOEWE programs Fh-TMP, NeFF and OSF as well as by the EU COST Actions BM0806, BM1007 and CM1103.

HISTAMINE H_4 RECEPTOR DRUG DISCOVERY: LESSONS LEARNED AND NEW OPPORTUNITIES

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Since the discovery of the H_4 receptor and the elucidation of their role in the modulation of the immune system, there has been considerable interest in the therapeutic potential of histamine H_4 receptor antagonists. Up to now, drug discovery efforts in this field have given some important insights into what the required pharmacological profiles of new H_4R ligands are, but also what they should probably not be. In this presentation a brief overview of success and failure in H_4R drug research will be given and the pre clinical drug discovery strategy of Griffin Discoveries will be discussed. In this discussion we will adress several important issues including H_4 receptor species differences, H_4 receptor residence time, HERG interaction to consider and what their impact is on our lead optimization programs. Moreover, we will discuss our strategy that aims to combine both H_4R and H_1R antagonism by a single molecule as an alternative to developing selective histamine H_4R antagonists.

DISCOVERY OF JNJ 39758979 AND SAR OF RELATED 2-AMINOPYRIMIDINE HISTAMINE H_4 ANTAGONISTS

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This report discloses the discovery and SAR of a series of 2-aminopyrimidine derived histamine H_4 antagonists including the clinical candidate JNJ 39758979. The initial histamine H_4 receptor high throughput screening campaign identified a tricyclic pyrimidine series of low nanomolar inhibitors. Though potent, this series suffered from rapid in-vitro metabolism in human and rodent assays as well as narrow SAR around the diamine component. Building on the SAR studies of saturated derivatives from the indole carboxaminde series, typified by JNJ 7777120, and incorporating knowledge from the tricyclic pyrimidines led us to the 2-aminopyrimidine series. A focused medicinal chemistry effort delivered several 6-alkyl-2,4-diaminopyrimidines that behaved as antagonists at both the human and rodent H_4 receptor. Further optimization led to a panel of antagonists that were profiled in several animal models of inflammatory disease. Based on the preclinical profile and efficacy in several animal models, JNJ 39758979 was selected as a clinical candidate. JNJ 39758979 was well tolerated in phase I human clinical trials and progressed into phase II clinical trials.

THE HISTAMINE 4 RECEPTOR INHIBITS HUMAN NEUTROPHIL PHAGOCYTOSIS

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Background: Neutrophils constitute the first line of defence against bacteria. Neutrophils kill pathogens by releasing antimicrobial peptides and proteases (degranulation) in the extracellular milieu or in the phagosome (phagocytosis). There is controversy as to whether the H_4R is expressed in human neutrophils and plays a role in the regulation of neutrophil functions.
Aims: To investigate whether the H_4R regulates antimicrobial functions of neutrophils.
Methods: Neutrophils were isolated from blood by centrifugation through Ficoll-Hypaque. Degranulation was assessed by measuring the release of lactoferrin in the extracellular milieu (ELISA). Phosphorylation of p38 MAP Kinase was measured by Western blot analysis. Rates of phagocytosis and bacterial killing were measured from the decrease in the number of extracellular bacteria and change in the number of intracellular bacteria.
Results: Histamine (10^{-9}-10^{-6} M) and the H_4R agonist JNJ 28610244 (10^{-7}-10^{-5} M) blocked in a dose-dependent manner the release of lactoferrin by adherent neutrophils stimulated with fMLP (0.1 μM). The inhibitory effect of histamine was reversed by cimetidine (H_2R antagonist) or JNJ 7777120 (H_4R antagonist), but not by ceterizine (H_1R antagonist). JNJ 28610244 inhibited activation of the p38 MAP kinase in adherent neutrophils stimulated with fMLP (0.1 μM). The rate constants for phagocytosis (uptake of bacteria) were: k_1 = 0.17 min^{-1} ($t_{1/2}$= 4 min) for control neutrophils; k_1 = 0.11 min^{-1} ($t_{1/2}$= 6 min) for neutrophils exposed to histamine (10^{-6} M) or JNJ 28610244 (10^{-5} M). The rate constants for bacterial killing were: k_2 = 0.14 min^{-1} ($t_{1/2}$= 5 min) for control neutrophils; k_2= 0.04 min^{-1} ($t_{1/2}$= 16 min) for neutrophils incubated with histamine (10^{-6} M); k_2= 0.08 min^{-1} ($t_{1/2}$= 9 min) for neutrophils incubated with JNJ 28610244 (10^{-5} M).
Conclusions: 1- Engagement of the H_4R impairs neutrophil phagocytosis through inhibition of p38 MAPK and degranulation.

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JNJ7777120 COMPOUND AMELIORATES DAMAGE IN SALIVARY GLANDS, GINGIVA AND PERIODONTAL BONE PRODUCED BY EXPERIMENTALLY INDUCED PERIODONTITIS IN RATS

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Periodontitis is a chronic disease caused by oral bacterial infection and persistent local inflammation, which results in alveolar bone resorption, soft tissue attachment injury and could lead to tooth loss. Recently, we have reported that histamine is able to reduce experimental periodontitis (EP)-induced damage on submandibular gland (SMG) and periodontal bone structure. This study investigates whether the H_4R ligand, JNJ7777120 (J77), could prevent 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. J77 treatment (10 mg/kg, s.c.) was started 5 days before the end of the experimental period.

J77 treatment prevented EP-induced SMG histological damage, reducing vacuolization and apoptosis (2.1±0.8 vs. 70.3 ± 3.4 apoptotic cells per field, $P<0.001$). In addition, J77 completely reversed the increased PGE2 levels in SMG (658.6 ± 160.6 vs. 1774.0 ± 158.8 fg PGE, $P<0.01$) while partially reversed the methacholine-induced salivation reduction produced by EP. The protective effect exerted by J77 on SMG functionality is associated with the attenuation of the lingual and vestibular bone loss determined as distances between the cemento-enamel junction and the alveolar crest (0.78 ± 0.05 vs. 1.02 ± 0.05 mm; $P<0.01$) and the reduction of the increased PGE2 (2217 ± 290 vs. 5972 ± 1602 pg PGE, $P<0.05$) and TNFα levels and inflammatory infiltrations in gingival tissue of rats with EP.

We conclude that J77 is able to ameliorate periodontitis-induced injury on SMG, gingival tissue and bone structure, suggesting that pharmacological targeting of H_4R could be an attractive strategy for the treatment of periodontal disease.

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THE EFFECTS OF THE H4R ANTAGONIST JNJ7777120 ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY HUMAN NEUTROPHILS

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There are 4 types of histamine receptors (H_1R, H_2R, H_3R, and H_4R), all belong to the G protein-coupled receptor family. Histamine has been shown to regulate the functions of different immune cells including neutrophils. Neutrophils are professional phagocytes of innate immune system. Upon activation they produce reactive oxygen species (ROS). Based on our previous studies with H_1R antihistamines (e.g. loratadine, dithiaden, pheniramines), we examined the effects of H_4R antagonist JNJ7777120 on ROS production in human neutrophils.

Using luminol-enhanced chemiluminescence we investigated the effects of JNJ7777120 (10^{-10} – 10^{-4} mol/l) on the production of ROS in activated neutrophils in whole blood. Four different stimuli were used to activate neutrophils: phorbol-myristate-acetate (PMA), opsonised zymosan (OZ), calcium ionophore (A23187) and formyl-methionyl-leucyl-phenylalanine (fMLP). Luminol plus superoxiddismutase/catalase or isoluminol were used to distinguish between intracellular and extracellular ROS production, respectively. The effects of JNJ7777120 on production of ROS vary depending on the stimuli and concentrations used: there was no effect of JNJ7777120 on PMA or OZ stimulated production of ROS. JNJ7777120 increased the ROS production after A23187 stimulation. In contrast, JNJ7777120 inhibited fMLP activated ROS production but this was not concentration dependent. JNJ7777120 inhibited both intracellular and extracellular ROS production. However JNJ7777120 did not scavenge ROS produced in the H_2O_2-luminol-horseradish peroxidase cell free system.

Different signalling pathways are involved in the activation of neutrophils by PMA, OZ, A23187 or fMLP. Our results encourage us to investigate the question why JNJ7777120 only inhibits fMLP induced ROS production in neutrophils.

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EFFECTS OF HISTAMINE AND ITS H4 RECEPTOR AGONISTS ON REACTIVE OXYGEN SPECIES PRODUCTION IN HUMAN LEUKOCYTES

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Histamine, an endogenous biogenic amine, is an important chemical messenger which has numerous physiological roles in central and peripheral tissues. These effects are mediated via four histamine receptors - H_1R, H_2R, H_3R and H_4R, which belong to the superfamily of G protein-coupled receptors. We investigated the effects of histamine and specific H_4R agonists 4-methylhistamine and VUF8430 on the production of reactive oxygen species (ROS) in human whole blood and isolated leukocytes.

Antioxidant parameters of histamine receptor agonists were investigated using total peroxyl radical-trapping antioxidant parameter analysis, oxygen radical absorbance capacity assay and NO-scavenging determination. Determination of ATP activity was used for evaluation of cell viability. The ability of isolated leukocytes or leukocytes in the whole blood of healthy human volunteers to produce ROS after histamine or its H_4R agonist treatment (10^{-8} – 10^{-4} M) was tested by luminol-enhanced chemiluminescence, spontaneous or activated by opsonised zymosan particles (OZP) or phorbol-myristate-acetate (PMA).

None of the studied compounds had any antioxidant potential against ROS. All three compounds significantly decreased the spontaneous and OZP-activated chemiluminescence response in whole blood leukocytes in a dose dependent manner. On the other hand, only VUF8430 was dose-dependently effective when whole blood leukocytes were activated with PMA. Generally, the effects of all three compounds were similar but less profound in isolated leukocytes.

It can be concluded from our results and the literature that the inhibition of ROS production by tested compounds was most probably caused by H_2R rather than by H_4R. Especially at high concentrations of histamine the signal is transduced mainly through H_2R and may inhibit the ROS production.

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ANTINOCICEPTIVE ACTION OF JNJ7777120 IN ACUTE PAIN MODELS AND ITS INTERACTIONS WITH ARACHIDONIC ACID DERIVATIVES INHIBITORS

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Until now only few studies demonstrated analgesic action of H_4 antagonists. However, there is no studies about mechanisms of its antinociceptive effect. It is known that hyperalgesic response in experimental inflammation is primarily dependent on arachidonic acid derivatives. That`s why our hypotheses assumes that effect of H_4R antagonist may be secondary connected with its anti-inflammatory action.

The aim of these study was to explain the mechanism of antinociceptive effect of H_4R. To verify hypothesis that observed antinociceptive effect of H_4R blockade is a result of regulation of synthesis of leukotrienes or synthesis of prostaglandins we compared analgesic activity of H_4R antagonist with 5-LOX, COX-1, COX-2 inhibitors in acute pain and acute inflammatory pain models.

The research has been conducted on a group of male Lewis rats weighting 250-350 g. Inflammation was induced by injection of 1% carrageenan into rats` hindpaw. The pain threshold levels were determined by using mechanical stimuli (the Randall - Selitto test) and thermal stimuli (Plantar test method). JNJ7777120 in the dose 25 mg/kg was administered intraperitoneally alone or in combinations with COX-1 inhibitor (indomethacin 3 mg/kg), COX-2 inhibitor (celecoxib 25 mg/kg), 5-LOX inhibitor (aesculetin 10 mg/kg). Pain threshold was assessed after 30 minutes and 1 hour after JNJ7777120 administration. After one hour animals were anesthetized and blood from heart was collected for morphological and biochemical analysis.

We have confirmed that H_4R antagonist JNJ7777120 exhibits antinociceptive effect in acute pain and in acute inflammatory pain models against mechanical stimuli. Moreover, concomitant administration of the H_4R antagonist and COX-1 inhibitor increased the pain threshold in rats.

THE ROLE OF HISTAMINE RECEPTORS ON GLUCAGON SECRETION IN ATC1.6 CELL

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The pancreatic islets of Langerhans play a pivotal role in glucose homeostasis by secreting insulin and glucagon. Several G protein-coupled receptors are involved in insulin secretion from pancreatic β-cells. We previously reported that histamine H_3 receptor (H_3) was expressed in pancreatic β-cells and it had an inhibitory effect on insulin secretion. Pancreatic islets are composed largely of β-cells, but also contain several different cells such as α-cells. The α-cells of pancreatic islets play an important role in glucose homeostasis as well as β-cells. Pancreatic α-cells secrete glucagon in response to low glucose concentration to restore hypoglycaemia. We found a clue that α-cells would also express histamine receptors in the previous study. However, the role histamine receptors on pancreatic α-cells remains to be elucidated. In the present study, we aimed to reveal the expression and function of histamine receptors in pancreatic α-cells. We found that histamine H_1 receptor (H_1) and H_3 were expressed in αTC1.6 cells, a cell line derived from a mouse pancreatic α-cell. Next, we examined the effects of histamine, H_1 and H_3 agonist on glucagon secretion from αTC1.6 cells. Selective H_1 agonist 2-pyridylethylamine increased glucagon secretion, whereas histamine and selective H_3 agonist immepip remarkably inhibited glucagon secretion in response to low concentration glucose stimulation. In addition, we found that 2-pyridylethylamine enhanced and immepip attenuated the increase of intracellular Ca^{2+} concentration, which was essential for glucagon secretion, in response to low concentration glucose stimulation. To summarize our data, H_1 and H_3 were expressed in αTC1.6 cells. H_1 agonist increased and H_3 decreased glucagon secretion. These results may suggest that histamine receptors are involved in the regulation of serum glucose concentration by modulating glucagon secretion from pancreatic α-cells.

EXPRESSION OF HUMAN HISTAMINE H_2-REGEPTORS IN TRANSGENIC MICE

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In many species, histamine can induce positive inotropic effects and positive chronotropic effects via stimulation of histamine H_2-receptors leading to an increase in intracellular cAMP content. In addition it is well accepted that histamine can also induce cardiac arrhythmias, also in isolated human cardiac preparations. However, a mouse model to study this system in more detail was hereby not available. Therefore, we generated transgenic mice (TG) with cardiac-specific overexpression of the human H_2-receptor under control of the α-myosin heavy chain promoter and compared them with wild type (WT) littermates. In isolated electrically paced (1 Hz) left atria and spontaneously beating right atria, histamine and dimaprit (a H_2 agonist) induced a concentration-dependent positive inotropic and chronotropic effect, respectively. All effects of histamine were noted only in TG preparations and not in WT controls and they were blocked in TG by cimetidine, a H_2-receptor antagonist. Furthermore, a higher incidence of histamine induced arrhythmias was noted in isolated right atrial preparations compared to WT. In vivo, using left ventricular echocardiography (isoflurane anesthesia), histamine and dimaprit induced positive inotropic and positive chronotropic effects only in TG and not in WT which were antagonized by cimetidine. A H_1-receptor antagonist (mepyramine) was not able to elicit effects of its own and could not attenuate the inotropic (increase in ejection fraction) or chronotropic effects in TG. In summary, we generated H_2-receptor expressing mice, in order to study the function of these receptors in the living animal.

CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR HUMAN HISTAMINE N-METHYLTRANSFERASE

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Many findings on histamine metabolism obtained in animal models have yet to be confirmed in humans, which is mostly due to the lack of proper tools for studying histamine formation and inactivation in man. The lack of suitable antibodies for the histamine inactivating enzyme histamine N-methyltransferase (HMT) has so far prevented the direct analysis of the HMT protein in man and other mammals. Therefore, we set out to produce highly sensitive and specific monoclonal antibodies for human and porcine HMT that facilitate the detection and quantitation of the protein at the cellular and subcellular level.

Complete cDNAs encoding human and porcine HMT were expressed as GST fusions in E. coli, purified to homogeneity and used for immunization of mice to obtain monoclonal antibodies. These antibodies were screened for specificity, sensitivity and species cross-reactivity by ELISA and immunoblotting. Suitable antibody clones were subsequently used to analyze the expression and localization of HMT in human and porcine tissue sections and homogenates by immunohistochemistry and immunoblotting. .

Six different monoclonal antibodies specific for human HMT and four different monoclonal antibodies specific for porcine HMT were obtained that can detect HMT with up to 50-fold greater sensitivity than the most sensitive enzymatic assays currently available. Using these antibodies allowed us to confirm the expression and cellular localization of HMT in various human and porcine tissues where the presence of the enzyme had previously been deduced from activity measurement and HMT mRNA analysis. Immunohistochemical staining clearly showed that HMT is a cytosolic protein, which is localized in specific cells of most mammalian tissues.

The new monoclonal antibodies not only allow a comprehensive quantitative evaluation of the expression of HMT at the cellular level in man and other mammals but will also facilitate sensitive analyses of disease associated alterations of this protein.

EOSINOPHIL PURIFICATION FROM PERIPHERAL BLOOD - STUDY OF DIFFERENT IMMUNOMAGNETIC CELL SORTING METHODS EFFICIENCY

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Eosinophils are pleiotropic, multifunctional leukocytes, involved in many different biological reactions. They are playing a key role in multicellular anti-parasites immune response. Those cells are also responsible for pathogenesis of allergy, asthma, gastrointestinal disorders and other inflammatory diseases (Hogan et al., 2006). Recent discoveries have proven that histamine as an intercellular mediator, activates eosinophils via histamine H_4 receptor. Yet, there are still few analyses performed on histamine H_4 receptor, mainly because of eosinophils population purity problems (Seifert et al., 2012). This issue is related to specific characteristics of isolation methods, which are relatively difficult to optimize due to small eosinophils presence (1-6%) among the white blood cells and their limited viability. The aim of presented study is to compare different methods of eosinophils purification from peripheral blood, via immunomagnetic cell sorting. Eosinophils are isolated from fresh human blood by Ficoll-Paque density gradient separation, followed by negative immunomagnetic cell sorting. Erythrocytes are eliminated from blood samples using one of the following techniques: red blood cells lysis in ammonium chloride solution, dextran sedimentation or erythrocytes hypotonic shock lysis in distilled water. Results of eosinophils purification are evaluated by flow cytometric analysis. The viability of cells are measured in trypan blue exclusion assay. Considered methods result in different eosinophils isolation efficiency and cells survivability. We observe that presence of trace erythrocytes in the samples before separation have severe impact on immunosorting result. Samples treated with ammonium chloride solution or dextran sedimentation characterized with small separation efficiency or limited cell viability. Therefore it seems that method with erythrocytes lysis in distilled water is the most suitable for eosinophils purification.

In frame of COST BM0806, BM1007.

RADIOPROTECTIVE EFFECT OF JNJ7777120 AGAINST CYTOTOXIC AND GENOTOXIC DAMAGE OF IONIZING RADIATION

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Radiotherapy is one of the most widely used local modality for the treatment of cancer. Despite its high therapeutic index, ionizing radiation can cause disabling normal tissue injury causing serious adverse effects to patients. We have previously reported the radioprotective effect of histamine on highly radiosensitive tissues.

In the present work we aimed to investigate the possible mechanisms responsible for the radioprotective effect of the H_4R ligand, JNJ7777120 (J77), evaluating its effect on reducing ionizing radiation-induced injury and genotoxic and oxidative damages in the rat small intestine and hematopoietic tissue. For that purpose, 40 rats were divided into 4 groups. J77 and J77-irradiated groups received a daily sc J77 injection (10 mg/kg) starting 24 h before irradiation. Irradiated groups received a single dose of 5 Gy on whole-body using Cesium-137 source and were sacrificed 3 or 30 days after irradiation. Tissues were removed, fixed, stained with hematoxylin and eosin or PAS staining and histological characteristics were evaluated. Proliferation, apoptosis and oxidative DNA markers were studied by immunohistochemistry, while micronucleus (MN) assay and cytogenetic analysis were performed to evaluate chromosomal damage. Thiobarbituric acid reactive substances (TBARS) and catalase activity were determined by spectrophotometric techniques.

Results indicate that J77 reduced the grade of aplasia, and substantially prevented ionizing radiation-induced bone marrow replacement by adipose tissue, preserving all medullar components. J77 decreased the percentage of MN formation (3% vs. 13%, $P<0.01$) and chromosomal aberrations (4% vs. 14%) in irradiated bone marrow. In addition, J77 reduced MN frequency in peripheral blood (270 ± 76 vs. 1490 ± 219, MN/10,000 erythrocytes, $P<0.01$). J77 reversed ionizing radiation-induced spleen wet weight reduction, histological damage and increased TBARS (0.16 ± 0.02 vs. 0.35 ± 0.05 nmol /mg of protein, $P<0.05$). Furthermore, J77 diminished intestinal mucosa atrophy, edema and preserved villi and the number of crypts after radiation exposure (240±8 vs. 165±10, $P<0.05$). This effect was associated to a reduced apoptosis and chromosomal damage in intestinal crypts. We conclude that J77 exhibits radioprotective effects against radiation induced cytotoxic and genotoxic damage in small intestine and hematopoietic tissues and thus, could be of clinical value for patients undergoing radiotherapy.

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

NORMAL FIBROBLASTS INDUCE A MESENCHYMAL PHENOTYPE IN MAMMARY EPITHELIAL TUMOR CELLS WHICH IS BLOCKED BY HISTAMINE TREATMENT OF FIBROBLASTS

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Interactions between neoplastic and stromal cells are crucial for tumor growth and also for defining the metastasic behavior. Previously, we determined that histamine exerts a direct action in normal human fibroblasts CCD-1059Sk modulating its proliferation, migration and gelatinolytic activity. Epithelial to mesenchymal transition (TEM) is a biological process for cancers to be more aggressive and invasive. As fibroblasts are the main cellular component in the stroma, we proposed to evaluate the mesenchymal phenotype induced in mammary epithelial tumor cells when they were cultured with the conditioned medium (CM) derived from normal fibroblasts untreated or treated with histamine (0.1 μM and 20 μM), studying the expression of epithelial (E-cadherin) and mesenchymal markers (β-catenin, a-smooth muscle actin), TGF-β1, gelatinolytic activity and cell migration. We employed the mammary epithelial cell lines MDA-MB-231 and MCF-7, and fibroblasts CCD-1059Sk. In MDA-MB-231 cells, CM from control fibroblasts (CM(+)) induced morphological changes characterized by spindle-shaped cells and scattered colonies. There was an increase in a-smooth muscle actin, β-catenin and TGF-β1 by Western blot, an increase in MMP2 activity by zymography (400% vs 100%(CM(-), $p<0.05$) and an enhancement of cell migration using transwells units (135% vs 100%(CM(-), $p<0.05$). In MCF-7 cells, CM(+) induced morphological changes in cells and colonies; there was no change in total protein levels of cell markers by Western blot but an altered subcellular distribution. Besides, no increase in MMP2 activity or cell migration was observed. Notably, the distinctive and the intermediate mesenchymal phenotype induced by CM(+) in MDA-MB-231 and MCF-7 cells respectively were reverted by CM from histamine-treated fibroblasts. In summary, HA might modify tumor microenvironment and therefore contribute to design future antineoplastic therapies.

P6

ANTIPROLIFERATIVE EFFECTS OF H_4R AGONISTS ON HUMAN WIDR COLORECTAL CANCER CELL LINE

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Colorectal cancer is one of the leading causes of cancer death among both men and women worldwide. It was reported that the levels of histamine are elevated in colon carcinoma tissue which is directly related to an increase in histidine decarboxylase activity and a decrease in diaminooxydase activity. It was also demonstrated that the levels of the H_4R are reduced in advanced colorectal cancer compared to an initiating state, which suggests that the H_4R expression is regulated during the progression of the disease. The aim of the present work was to investigate the role of H_4R agonists on the proliferative potential of human WiDr colorectal adenocarcinoma cell line. For that purpose, we evaluated cell proliferation by the clonogenic assay, and the incorporation of BrdU using H_4R agonists [Histamine (HA), Clobenpropit (Clob), Clozapine (CLZ), JNJ28610244 (J28), and VUF8430 (VUF)] and the antagonist (JNJ7777120, J77). Apoptosis was studied by Annexin-V staining and flow cytometry, and TUNEL assay while reactive oxygen species (ROS) were evaluated by flow cytometry.

H_4R agonists significantly decreased proliferation of WiDr cells with a half maximal inhibitory concentration (IC_{50}) of 1.1±0.7 μM for CLZ; 1.8±0.7 μM for Clob; 1.9±0.6 μM for J28. This effect was associated with a reduction in BrdU incorporation, an augment in TUNEL positive cells ($P<0.01$), and a 2 fold increase in ROS levels. All H_4R agonists were more effective in inhibiting proliferation than histamine. No mitogenic activity was observed after treatment with H_1R (2-(3-(trifluoromethyl)phenyl)histamine), H_2R (anthamine) or H_3R (R(-)-α-methylhistamine) agonists.

We conclude that in vitro findings show that H_4R agonists can inhibit colon cancer cell proliferation and thus could be considered for further preclinical studies.

We thank Dr. Nicholas Carruthers from Johnson & Johnson Pharmaceutical Research & Development for the JNJ28610244 and JNJ7777120 compounds.

H_4R AGONISTS SUPPRESS HUMAN MELANOMA GROWTH AND LUNG METASTASES

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Melanoma arises from epidermal melanocytes and is a major concern for health-care providers. It has been previously reported the expression of H_1R, H_2R, H_3R and H_4R in human primary (WM35) and metastatic (M1/15) melanoma cell lines. In these cell lines, histamine through the H_4R decreases proliferation, inducing melanogenesis and senescence. Histamine and clozapine also show in vivo antitumor activity on human M1/15 melanoma xenografts. The aims of this work were: to investigate signal transduction pathways and biological responses triggered by the activation of H_4R in human highly invasive and metastatic 1205Lu melanoma cells and to evaluate the in vivo antitumor activity of the H_4R agonists histamine (HA), clozapine (CLZ) and JNJ28610244 (J28). Results indicate that 1205Lu cells express H_4R determined by RT-PCR and western blot. H_4R agonists increased phosphorylation levels of ERK1/2 while significantly decreased clonogenic proliferation of 1205Lu cells with a half maximal inhibitory concentration (IC_{50}) of 1.6 μM for histamine and 10 μM for J28. The latter effect was associated with an enhanced cell differentiation. In vivo studies show that HA, CLZ and J28 (1 mg/kg-1, sc) significantly decreased tumor growth of 1205Lu melanoma xenografts developed in nude mice ($P<0.001$). This was related to a reduction in the mitotic index (9.2±1.5 for HA, 11.8±2.6 for CLZ, 10.6±2.1 for J28 vs. 19.2±1.6 for untreated mice, $P<0.01$), and in the intratumoral neovascularization. Importantly, the metastatic spread of the tumors to the lung was completely prevented by J28 treatment. We conclude that H_4R agonists exhibit an antitumoral effect in vitro and in vivo on metastatic melanoma, suggesting the therapeutic potential of these compounds for the treatment of melanoma.

We thank Dr. Nicholas Carruthers from Johnson & Johnson Pharmaceutical Research & Development for the JNJ28610244 compound.

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EXPRESSION PATTERN OF HISTAMINE-RELATED GENES IN ENDOMETRIAL CANCER

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Endometrial cancer is the second of most common gynecological cancer in Poland with the peak incidence at the age of 55-69. Two types of endometrial cancer, type I (estrogen-related, endometrioid endometrial cancer) and type II (estrogen-unrelated), may be distinguished on the basis of molecular and pathological characteristics. Endometrial tumorigenesis is still poorly understood. It has been postulated that histamine plays a role in development of gynaecologic cancer. The identification of genes and biochemical pathways is important for understanding the biology of endometrial tumorigenesis and progression.

The aim of this study was to estimate distinguished genes of histamine-related pattern in endometrial cancer in different pathomorphological stages (G1,G2,G3). Human endometrial tissues were obtained from patients treated in the Department and Clinic of Gynecology and Obstetrics, the Medical University of Silesia, Katowice. Gene expression profiles of twenty four samples were analyzed using oligonucleotide microarrays of HG-U133A (Affymetrix), enabling analysis of 22283 mRNA transcripts. From which we selected 119 histamine-related genes were analyzed them statistically by the Gene Spring 11.5 software. The level of significance was set at $P \leq 0.05$.

To identify genes associated with the progression of endometrioid cancer, we compared the expression profiles of genes in stages G1, G2 and G3. On the basis of supervised analysis identified ten different genes (LASP1, ITPKB, SLC22A3, CPA3, DRD2, LILRA2, RASA4, DRD2, RAB25, HRH3), which distinguished the endometrial cancer tissues from normal endometria. Additionally, we found that histamine H_3 receptor expression is increased with pathomorphological stages of endometrial cancer.

We can conclude that H_3 receptor gene may be regarded as potential marker for endometrial cancer progression.

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COMPARISON OF GENE EXPRESSION PROFILES CONNECTED WITH PROLIFERATION PROCESS IN NORMAL AND ENDOMETRIAL CANCER CELLS

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Processes such as cell proliferation, cell cycle progression, differentiation and apoptosis in both normal and cancer cell are influenced by histamine and histamine receptors. In human breast cells, normal and cancer, in different neoplasias like melanoma and colon cancer the high expression of H_3 or H_4 receptors have been postulated to behave as an important regulator of proliferation. In recent our research we found that H_3 receptor play a crucial role in endometrial cancer. Thus, the aim of this study was to compare the expression profile of genes connected with proliferation process in normal endometrial tissue and endometrial cancer.

The intact slices of endometria and endometrioid adenocarcinoma from postmenopausal (61 ± 5 years) women were collected in the Department of Gynecology and Obstetrics, Medical University of Silesia and for this received approval of ethic committee. RNA was isolated and cleaned up with RNeasy Kit (Qiagen) and the quantities and qualities were estimated by spectrophotometry and electrophoresis. GeneChip arrays (Affymetrix Co.) were performed according to the manufacturer's instruction. Obtained fluorescence signals were analyzed statistically using Gene Spring 11.5 software package.

Among the probes placed on the HG-U 133A microarray distinguished 7309 related to the proliferation process. Comparing their relation in different grading of endometrial cancer with controls the differences for the 717 transcripts at $p \leq 0.01$ were statistically significant. Due to the large number of results for significant p-value and fold change parameter we used an correction analysis and limited them to 21 genes.

Based on the obtained results it can be concluded that the fold change parameter for differentiating genes increased with grade of endometrial cancer.

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TGF-β GENES INVOLVED IN CELL CYCLE REGULATION IN ENDOMETRIAL CANCER

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TGF-β is a cytokine that effects functioning and cellular interaction in a particular way. Through effective cooperation with other proteins, stimulating them as a cascade and TGF-β revealing its pleiotropic function. Recent studies show that TGF-β controls the cellular processes such as: cell division, differentiation or homeostasis. The main purpose of this study was to select between genes involved in cell cycle regulation and TGF-β that undergo in pathological stages compared to healthy tissues which will determine characteristic expression profile for defining pathology and becoming a valuable diagnostic reference in identification of endometrial cancer progress.

The analyses were carried out on endometrial cancer samples collected from patients during surgery. Samples were collected from the central part of the tumor in different disease stages - histopathologically classified as adenocarcinoma and from the control - histopathologically correct tissue. From all of them complete RNA was extracted by the use of TRIZOL® reagent. The obtained cRNA was hybridized with HG-U133A microarrays (Affymetrix).

From 22283 obtained transcripts, 1050 were associated with TGF-β and 126 of them participated in cell cycle regulation. With the use of Gene Spring GX program these selected genes were analyzed statistically and visualized by box whisker, profile and volcano plot. Controls were compared to the three different stages of investigated cancer.

The healthy tissue was differentiated by the five of G1 and fifteen of G2 and eight of G3 genes associated with TGF-β activity which regulate cell cycle. The genes KATNB1, RAB38, PTEN, NRCAM, CDK14, MBTD1, PPP1R13B, STYXL1, ACTR2, RAB13, STAG1, TOB1, PIN1, LZTR1, FGF2, PTPN14, TP53, PML, FKBP1A, RAB25 are specific for the different disease stages. All of them are statistically significant and may represent as markers differing severity of endometrial cancer.

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AMINOOXY ANALOGUE OF HISTAMINE IS AN EFFICIENT INHIBITOR OF MAMMALIAN L-HISTIDINE DECARBOXYLASE: COMBINED IN SILICO AND EXPERIMENTAL EVIDENCES

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Histamine plays highlighted roles in the development of many inflammatory, neurological emergent and rare diseases [1]. At present, the regulation of the effects exerted by histamine is currently achieved by using modulators of the activities of the four histamine G-protein-coupled receptors, namely H_1 to H_4. Histamine is formed decarboxylation of the amino acid L-histidine, which is catalyzed by pyridoxal-5'-phosphate (PLP) dependent histidine decarboxylase (HDC, EC 4.1.1.22). The reduced availability and stability of the protein have delayed the characterization of its structure-function relationships. It has been a handicap to design intervention strategies based on histamine synthesis inhibition [2].

HDC could be an interesting target for intervention of multiple human diseases [1]. Our previous knowledge (derived from both in silico [2] and experimental approaches [3] on rat HDC) encouraged us to disclose some structure-activity relationships being of importance for design of novel specific inhibitors of the enzyme.

Our in silico (virtual screening) results indicated that an effective competitive inhibitor should be capable to form an "external aldimine-like structure" and to have an imidazole group, or its proper mimetic, to provide additional affinity of PLP-inhibitor adduct to HDC active center. Now this is confirmed with the experiments with aminooxy analogues of histamine, being capable to form PLP-oxime in the enzyme active center that resulted in promising lead compound. Taken advantage of the available mammalian HDC models [4,5], we have also determined the interactions that could stabilize the required conformation of this PLP-oxime, mimicking the external aldimine in the active site of mammalian HDC.

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References

1. *Pino-Ángeles A, Reyes-Palomares A, Melgarejo E, Sánchez-Jiménez F. J Cell Mol Med. 2012, 16: 1947-60.*
2. *Moya-García AA, Pino-Ángeles A, Gil-Redondo R, Morreale A, Sánchez-Jiménez F. Br J Pharmacol. 2009; 157: 4-13.*
3. *Rodríguez-Caso C, Rodríguez-Agudo D, Moya-García AA. Eur J Biochem. 2003, 270: 4376-87.*
4. *Moya-García AA, Ruiz-Pernía J, Martí S, Sánchez-Jiménez F, Tuñón I. J Biol Chem. 2008, 283: 12393-401.*
5. *Komori H, Nitta Y, Ueno H, Higuchi Y. J Biol Chem. 2012, 287: 29175-83.*

CLINICAL SIGNIFICANCE OF HISTAMINE H_1 RECEPTOR-PKC DELTA-HSP90 SIGNALING IN ALLERGIC SYMPTOMS

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Stimulation of histamine H_1 receptor (H_1R) leads to the increase of H_1R-mediated signaling which induces H_1R up-regulation through the elevation of H_1R gene expression via PKCδ activation. A positive correlation between symptoms and H1R mRNA in nasal mucosa was observed in both pollenosis patients and nasal hypersensitivity model rats. This evidence suggests that H_1R is an allergic disease sensitive gene.

Symptoms of nasal hypersensitivity model rats were improved with antihistamines or Kujin, an anti-allergic Kampo medicine. Nasal H_1R mRNA elevation was also suppressed by these drugs. After this, we elucidated the mechanism of H_1R gene expression activated by H_1R stimulation and suppressed by these anti-allergic drugs, and attempted to demonstrate the clinical significance of H_1R gene expression.

HeLa cells expressing endogenous H_1Rs and nasal hypersensitivity model rats were used for the proposed study. Determination of mRNA was performed by real-time quantitative RT-PCR. Signaling molecules and their phosphorylation were analyzed by immunoblot analysis, and their subcellular localization was performed immunocytochemically using confocal microscope.

Histamine- and PMA-induced H_1R mRNA elevation in HeLa cells was suppressed by rottlerin, a PKCδ inhibitor, U0126, a MEK inhibitor. and DPG, a PARP inhibitor. This elevation was also suppressed by Kujin. (-)Maackiain was identified as an active constituent of Kujin, and suppressed PKCδ activity. The target of (-)maackiain was identified to be HSP90. (-)Maackiain dissociated HSP90-PKCδ complex. Celastrol, a HSP90 inhibitor, improved symptoms of nasal hypersensitivity rats as well as showed a correlative improvement on mRNA elevation.

H_1R-mediated activation of H_1R gene expression is suppressed by antihistamines and (-)maackiain at the different level. H_1R-mediated allergic symptoms are correlated with PKCδ-mediated elevation of H_1R gene expression. HSP90 is a potential target of therapeutics for allergic diseases.

EFFECTS OF A SELECTIVE INHIBITOR POLY (ADP-RIBOSE)POLYMERASE (PARP) ON MAST CELL ACTIVATION BRONCHO-CONSTRICTION AND LUNG FIBROSIS

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Poly(ADP-ribose)polymerases (PARPs) are a family of enzymes that catalyzes the attachment of PAR from NAD to target proteins. PARP-1, the most abundant of these proteins, modulates the expression of a number of inflammatory genes. Here we studied the effects of hydroxyl-dimethylaminomethyl-thieno[2,3-c]isoquinolin-5(4H)-one (HYDAMTIQ), a PARP-1 inhibitor, in animal models of asthma-like reaction, in the guinea pig, and of bleomicin-induced lung fibrosis, in mice. Ovalbumin (OA)-sensitized guinea pigs, treated i.p. for 7 days with HYDAMTIQ (1, 3 and 10 mg/kg b. wt), were challenged with OA (5 mg/ml) for 60 sec. and respiratory parameters recorded. After 48 h the animals were anesthetized and challenged with MeCh (0.1 mg/ml) for 60 sec. Changes in the pressure at the airway opening (PAO) were registered. At the end, bronchial airway liquid (BAL) was collected for PAR expression. and lung removed for the evaluation of eosinophil infiltration, mast cell degranulation, prostanoid and cytokine production. C57/bl6 mice, treated with bleomicin, received for 21 days HYDAMTIQ (3 and 10 mg/kg b. wt.). PAO was assayed and lung tissue processed to evaluate the production of oxidative stress, inflammatory and fibrotic markers as well as percentage of positive goblet cells and thickness of smooth-muscle layer. Our results indicate that HYDAMTIQ exerts, in both experimental models, an anti-inflammatory and anti-fibrotic effect, as shown by the significant decrease of eosinophil and neutrophil infiltration, inflammatory cytokine and prostaglandin production. Moreover, HYDAMTIQ also reduces goblet cell relative number, thickness of smooth-muscle layer, pro-fibrotic cytokine and collagen deposition. These effects are accompanied by a decrease of PAO, mast cell degranulation and histamine release.

The present findings suggest that PARP inhibitors could be a promising approach to alleviate lung inflammatory and fibrotic diseases and could provide background for future clinical trials.

EVIDENCE FOR "NATURAL" AND "INDUCED" MURINE BASOPHILS DIFFERING IN CALCIUM-DEPENDENT RESPONSIVENESS TO FcεRI CROSSLINKING IN TERMS OF HISTAMINE AND CYTOKINE PRODUCTION: ROLE OF AUTOCRINE IL-3

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It is widely acknowledged that basophils leave the bone marrow as fully differentiated immunocompetent cells. Herein we describe two functionally distinct populations of this lineage, with differential responsiveness to FcεRI crosslinking, depending on their prior exposure to IL-3 in vitro or in vivo. Stimulated basophils synthesized histamine together with IL-4 and IL-6, whether they were sorted ex vivo from freshly isolated bone marrow cells (resident basophils) or derived from cultures with IL-3 (BMDB, Bone Marrow Derived Basophils). However, the latter generated much more histamine, IL-4 and IL-6 as well as IL-13 and GM-CSF that were not detected in supernatants from resident medullary basophils in the same conditions. The increased responsiveness of BMDB depended on calcium signaling through calcineurin and Store-Operated calcium Channels (SOCs). It could be acquired by freshly isolated basophils through exposure to IL-3, which initiated an autocrine loop of endogenous IL-3 production in response to FcεRI crosslinking. Such an endogenous IL-3 production mediates the increase in histamine and cytokine productions as well as basophil survival as shown by the effect of anti-IL-3 antibodies during FcεRI stimulation. In conclusion, we postulate that basophils exist at least in two forms, namely a weakly FcεRI crosslinkingresponsive resident (or "natural") and a fully functional "induced" population.

MAST CELL BIOLOGICAL RESPONSES CAN BE AFFECTED BY IgE ALONE

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FcεRI cross-linking with IgE and antigen results in mast cell activation. It has been recently suggested that mast cell activity might be modulated via IgE by itself. However, much still remains to be understood concerning this remarkable phenomenon. Therefore, in the present study we examined the influence of IgE alone on various mast cell effector functions. The study was carried out in vitro on mature rat peritoneal mast cells. The effect of IgE alone, used at different concentrations (0.1-5 μg/mL), on mast cell degranulation and histamine release (spectrofluorometric method), cysteinyl leukotrienes (cysLTs) and TNF (ELISA) synthesis was examined. Moreover, we assessed FcεRI expression (flow cytometry) and migratory response (Boyden microchamber assay) of mast cells primed with IgE at low (1 μg/mL) and high (5 μg/mL) concentrations. It was noticed that IgE by itself triggered mast cells to preformed mediator secretion and de novo-synthesized cysLTs and TNF release, however, only when employed at concentrations ≥ 3 μg/mL. What is more, IgE alone was found to up-regulate FcεRI level. We also observed strong increase in spontaneous as well as TNF- and CCL5-induced migration of IgE-primed (5 μg/mL) mast cells, as compared with migratory response of non-primed mast cells. Molecular basis investigation using various specific inhibitors revealed that MAP kinases i.e. Erk and p38, Src and PI3 kinases as well as PLC/A2 were entirely or partially involved in IgE-dependent mast cell response. In summary, these results indicated that IgE by itself can mediate preformed and de novo-synthesized MC mediator release and can augment surface FcεRI expression. IgE alone also strongly influences migration of mast cells. Thus, our findings suggest that IgE can be one of the important players in regulation of mast cell activity and functioning within tissues.

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THE ANALYSIS OF MAST CELL AND EOSINOPHILIC INFILTRATES PRESENCE IN THE COURSE OF INFLAMMATORY BOWEL DISEASE

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Up to date several authors discusssed interactions between cells forming inflammatory infiltrates in the course of IBD, mainly dealing with endoscopic biopsy specimens. These usually contain only mucosa. We have evaluated full bowel wall sections, which seems to be especially important in patients with Lesniowski - Crohn's disease

The purpose of our study was to evaluate relationship between clinical course and apoptotic activity, highlighting the role of eosinophils and mast cells in patients with inflammatory bowel disease (IBD).

Full-thickness tissue fragments of intestinal wall taken from patients after colectomy were analysed. Patients were divided into three main groups: 1. Crohn's disease (CD) 2. ulcerative colitis (CU) 3. control. CU and CD groups consisted of patients who received surgical treatment in the course of IBD. Control group was formed of patients who underwent colectomy due to non-IBD disorders.

Tissue specimens were collected during standard grossing procedures. Each group consisted of 50 patients. All tissue fragments were paraffin embedded and stained histochemically and immunohistochemically. We have used antibodies against tryptase and CD31. We have also used specfic stainig methods to show presence of eosinophilic infiltrates and apoptotic cells. Slides from each case were independently examined by two experienced pathologists with the use of morphometrics workstation based on Leica QWin and ImageJ software. In every case the diagnosis was confirmed, All data were analysed in correlation to clinical information, to show influence on the course of the disease.

We've described the localization of eosinophilic and mast cell infiltrates in correlation apoptotic activity in tissue fragments. Our study confirmed previous reports on increased mast presence in IBD (especially in muscularis propria and subserosa layers in CD). We have also confirmed higher mucosal apoptotic activity, when compared to control and increased eosinophil presence in IBD.

This research was supported by a grant from the Center for Medical Postgraduate Education, under contract # 501-1-09-12-12.

HISTAMINE AND TOLL-LIKE RECEPTOR EXPRESSION ARE ALTERED IN PBMCs FROM INFLAMMATORY BOWEL DISEASE PATIENTS

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Histamine is a key mediator in both immediate type hypersensitivity reaction and chronic inflammatory responses. Toll-like receptors (TLR) are important players in innate immune response thanks to recognition of pathogen associated molecular patterns.

The aim of the project was to investigate the effects of histamine on TLR-stimulated PBMCs isolated from patients suffering from inflammatory bowel disease (IBD).

Fourteen patients diagnosed with IBD (7 with Crohn's disease and 7 with ulcerative colitis) and 6 healthy volunteers were included in the study. Expression of histamine receptors (H_1, H_2, H_4R) as well as TLR-2, TLR-4 and TLR-9 was investigated using REAL-TIME PCR. Cytokine levels (IL-12, TNFα, IP-10) were determined in culture supernatants after 24-hour stimulation in the presence or absence of histamine.

Significant differences in H_1R, H_2R, H_4R and TLR-2, TLR-4, TLR-9 gene expression were observed in patients with IBD compared to healthy volunteers. PBMCs from IBD patients secreted significantly more pro-inflammatory cytokines, compared to healthy volunteers, after TLR stimulation. Within the IBD group, cytokine secretion was higher from patients with CD compared to patients with UC. In the presence of histamine, TLR-stimulated pro-inflammatory cytokine secretion from PBMCs was significantly reduced for both IBD patients and healthy volunteers.

Patients with IBD display dysregulated expression of TLRs and histamine receptors. Histamine modulates the innate immune response in IBD patients and healthy subjects by inhibiting of the secretion of pro-inflammatory cytokines. Further examination of this anti-inflammatory effect may contribute to the development of new therapeutic strategies in IBD patients.

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THE INFLUENCE OF HISTAMINE-RELATED GENES ON INFLAMMATION

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One of the most known risk factors of cancer is inflammation. The effect of histamine and its receptors on causing inflammation has been very well documented but the role in colorectal cancer is not yet explained. From all four histamine receptors H_1, H_2, H_3, H_4 at least HRH-1 and HRH-2 have been correlated with tumor growth. The aim of these study was to check the correlation between expression of histamine-related and inflammatory genes in endometrial cancer.

The analyses were made on endometrial cancer samples collected from patients during surgery, from the central part of the tumor and were histopathologically classified accordingly to WHO standards, the control was taken from the healthy tissues and also histopathologically checked. From all of this samples total RNA was extracted by the use of TRIZOL® reagent. The obtained cRNA was hybridized with HGU 133A microarrays (Affymetrix).

From among the 22283 obtained transcripts we selected 119 correlated with histamine and 627 with inflammation. Statistical analysis was performed with the use of Gene Spring GX program.

After estimating the expression of histamine receptors in different grading tissues the difference between them and inflammation-related genes were compared. Approximately thirty genes connected with inflammation showed a statistically significant correlation with the H_3 receptor. These genes belong to different expression pattern, and have different biochemical, as well as physiological properties.

The study was supported by Medical University of Silesia grant No. KNW-1-076/D/1/0, KNW-1-016/D/0.

THE EXPRESSION OF HISTAMINE AND OTHER KEY MARKERS IN THE ZEBRAFISH GUT

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In the gastrointestinal (GI) tract of many vertebrates, histamine is synthesized by histidine decarboxylase enzyme (hdc) and stored in various cell types, to play a role in acid secretion, mucosal defence, fluid transport, neurotransmission, inflammation, immunity and carcinogenesis. Its biological functions are mediated by the four known histamine receptor subtypes.

The zebrafish (Danio rerio), a teleost fish belonging to the family of Cyprinidae, has been widely used as a model organism. The wall of the GI tract of zebrafish shows a high degree of similarity to that of mammals. However, little is known about the expression of histamine-related genes and other key markers of histaminergic functions in the zebrafish gut.

The present study aims to provide background information on some enteric neurons and markers as well as gene expression of histamine receptors in the intestine of the adult zebrafish.

Double staining on zebrafish adult intestine whole mounts and sections were carried out. Antibodies against neuronal protein C/D (HuC/D) and a neurotransmitter-specific primary antiserum (anti-histamine, anti-serotonin, anti-galanin and anti-neuropeptide Y) together with appropriate secondary antibodies were used in the study. Using in situ hybridization method (ISH), the types of cells expressing hdc and/or histamine receptor-like genes in adult zebrafish gut was analyzed. Digoxigenin-labeled probes were used, and the reaction product was made visible with antibodies against digoxigenin.

Immunofluorescence showed histamine-like immunoreactivity in the gut epithelium. Preabsorption tests (histamine-sKLH and histamine-sOVA as blocking reagents) confirmed the specifity of the staining. Yet, the presence of hdc needs to be confirmed with both PCR and of histamine with chromatographic methods. ISH for either histamine-like receptors or hdc has not given any positive results so far. The presence of serotonin positive nerve fibres and cell bodies was confirmed. Immunofluorescence revealed galanin and neuropeptide Y positive nerve fibres within the myenteric plexus as well as endocrine cells containing these peptides. ISH confirmed the presence of galanin in the GI wall.

Our results give more insight into the presence of key neurotransmitters and biogenic amines involved in maintaining the GI homeostasis.

Supported by a STSM from COST Action BM0806.

MAST CELLS GENERATE CYSTEINYL LEUKOTRIENES AND EXHIBIT ALTERED IgE-DEPENDENT RELEASABILITY UPON TLR3- AND TLR7-MEDIATED ACTIVATION

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Mast cells (MCs) are extremely numerous in the skin and mucosal surfaces, virtually at the portals of infection. Furthermore, these cells express functional Toll-like receptors (TLRs) detecting bacteria that enter the host. A few recent findings reported that MCs also possess intracellular TLRs, such as TLR3 and TLR7/8, recognizing dsRNA and ssRNA, respectively - common by-products of viral replication. Accordingly, it might be assumed that MCs participate in anti-viral defense or/and in patomechanisms of viral-related diseases. Therefore, the purpose of the research was to examine MC response to TLR3 and TLR7 ligands, i.e. poly(I:C) and R848, respectively. Bearing in mind that MCs play an essential role in allergic reactions, we also examined whether TLR3- and TLR7-depended activation affects IgE-mediated MC response. Experiments were carried out in vitro on freshly isolated fully mature rat peritoneal MCs (rPMCs). TLR3 and TLR7 protein expression was analyzed by western blot and flow cytometry, degranulation was evaluated by histamine release using spectrofluorometric method, cysteinyl leukotrienes (cysLTs) and CXCL8 generation was studied by ELISA tests. Our results showed that rPMCs constitutively express TLR3 and TLR7 molecules and selectively respond to poly(I:C) and R848 activation. TLR3- and TLR7-mediated MC stimulation led to dose- and time-dependent cysLT secretion without histamine release and CXCL8 generation. Moreover, we observed an augmented cysLT synthesis following MC co-stimulation with poly(I:C) and anti-IgE, whereas MC priming with R848 caused a reduction in anti-IgE-induced histamine release. Our results imply that dsRNA and ssRNA viruses can directly induce potent proinflammatory mediator release from mast cells and indirectly modulate IgE-dependent allergic processes.

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HOST DEFENSE PEPTIDE CATHELICIDIN LL-37 AS MAST CELL STIMULUS

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Cathelicidins represent a family of cationic peptides involved in innate host defense against pathogens, notably against bacteria. Apart from direct capacity to eliminate invading microbes, cathelicidins can also indirectly regulate immune response via affecting activity of cells involved in antibacterial defense mechanisms. Taking into account important role of mast cells in protection against bacterial infection, the aim of this study was to determine whether LL-37, human-derived cathelicidin, can influence mast cell response. We examined LL-37-mediated degranulation and histamine release (spectrofluorometric method), cysteinyl leukotriene (cysLT) generation, as well as CXCL8 and TNF synthesis (ELISA tests). LL-37-induced mast cell migration was also estimated (Boyden microchamber assay). Experiments were performed in vitro on mature connective tissue rat peritoneal mast cells. We have documented that mast cell activation with LL-37 resulted in dose- and time-dependent mast cell degranulation and histamine release, but failed to induce cysLT generation and release. What is more, stimulation of mast cells with LL-37 led to TNF, but not CXCL8, synthesis. We also stated that LL-37 induced mast cell migration. LL-37-mediated mast cell response was observed when cathelicidin was used at concentrations of ≥ 20 μg/mL, i.e. significantly higher than physiological level. To delve into LL-37-induced signal transduction pathways in mast cells, we examined the effect of the several inhibitors, such as PD98059 and SB203580 - MAPK pathway inhibitors specific to ERK and p38 molecules, respectively, U-73122 - an inhibitor of PLC/PLA_2, and LY294200 - an inhibitor of PI3K. Our results indicate that cathelicidins may augment antimicrobial inflammatory response by attracting mast cells to site of bacterial entry and by induction of mast cell-derived mediator release.

This work was supported by the Medical University of Lodz (Grant No. 502-03/6-164-01/502-64-004).

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PRELIMINARY CHARACTERIZATION OF HISTAMINE RECEPTOR EXPRESSION IN HUMAN LUNG MAST CELLS

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To date, four histamine receptors have been identified, H_1, H_2, H_3 and H_4 with variable expression depending on organ and species. While both H_1 antihistamines and H_2 antagonists are widely used in the treatment of allergy and gastrointestinal disorders, the potential therapeutic value of targeting H_3 and H_4 receptors is yet to be fully determined [1]. Previous studies have identified the expression of H_2 and H_4 receptors on human skin mast cells [2]. To our knowledge, histamine receptor expression has not been studied in human lung mast cells (HLMC). Therefore, the aim of this study was to determine which histamine receptors are expressed by HLMC.

HLMC were generated by physical and enzymatic disruption of lung tissue obtained from surgical resections. HLMC were initially purified by Percoll density gradient centrifugation followed by immunomagnetic cell separation (purity ≥ 95%). RNA was extracted from purified HLMC using TRI Reagent, cDNA synthesised and then amplified using primers specific to regions of the human histamine receptor subtypes [2,3]. Expression of β-actin was also assessed. Resulting PCR products were subjected to in-house genotypic analysis by automated sequencing. Expression levels of histamine receptors were analysed by image analyzer software (ImageJ v1.46). This study was approved by the National Research Ethic's Committee.

HLMC expressed H_1, H_2 and H_4 receptors but not H_3 at the mRNA level. The human mast cell line, LAD2, showed a similar profile of expression but H_2 receptor expression was approximately 6 fold lower. Pharmacological characterization of histamine receptors in human lung mast cells is currently being studied.

These preliminary studies indicate that both HLMC and LAD2 cells express H_1, H_2 and H_4 receptors at the mRNA level.

References

1. *Zampeli E, Tiligada E. Br J Pharmacol. 2009; 157: 24-33.*
2. *Lippert U, Artuc M, Grützkau A. Et al. J Invest Dermatol. 2004; 123: 116-23.*
3. *Wang KY, Arima N, Higuchi S et al. FEBS Lett. 2000; 473: 345-8.*

EFFECT OF HISTAMINE ON HUMAN EPITHELIAL CELL LINES

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We have previously shown that histamine stimulates IL-6 and IL-8 release in cystic fibrosis (CFBE) but not normal epithelial cells (HBE) .and found immunocytochemical evidence for the presence of the H_4 receptor on both cell types. The aim of this study is to determine if histamine activates volume regulated anion channel (VRAC) currents in HBE and CFBE cells and to further characterise the expression of histamine receptors in these cells.

The presence of VRAC was examined in CFBE and HBE using patch clamp methods. The effect of histamine and histamine receptor antagonists on the current was tested. All 4 histamine receptors were studied in both HBE and CFBE cells by immunocytochemistry, western blot and real-time PCR.

Histamine activated a chloride current, indicative of the VRAC current. The current density was significantly reduced in CFBE cells. In HBE cells the current was significantly inhibited by cimetidine, but not by the diphenhydramine hydrochloride, methylhistamine dihydrochloride or JNJ7777120. In CFBE cells this same trend was repeated although the action of cimetidine was not significant. Positive staining for all four receptors was found and confirmed by western blot analysis. The protein bands for the H_2R and H_3R subtypes appeared reduced in CFBE cells in comparison to the HBE cells. Real-time PCR confirmed the presence of all receptor subtypes, but with an apparently reduced expression of H_2R and H_3R in CFBE cells.

Histamine has previously been shown to activate VRAC in cell types such as human keratinocytes; we demonstrated that histamine can activate the VRAC current in both HBE and CFBE cells. However, this activation is significantly decreased in CFBE cells. The histamine activated VRAC current could be significantly inhibited by the H_2R antagonist cimetidine, but not by the H_1R, H_3R or H_4R antagonists in HBE cells even though all 4 histamine receptors are present.

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EFFECTIVENESS OF PHARMACOLOGICALLY INDUCED HISTAMINE RELEASE IN THE TREATMENT OF COMMON DISEASES IN DOGS

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Histamine is a key immunomediator in the antiviral response. Host-virus interactions result in local histamine release in the infected tissue, which is often responsible for the clinical symptoms.
Basophile leukocytes and mastocytes are the main effectors of the hypersensitivity mediated by immunoglobulin E, or the type I, and they have an important role in protection against viral infections.
The benefits of this histamine release are not well understood, but there is some clinical and laboratory evidence suggesting a direct antiviral effect of histamine.
The diseases shown in this work are viral respiratory infection (canine distemper), viral gastroenteritis and canine papillomatosis.
The drugs used are: lacto-therapy (through the use of Tindalac), oxitetracycline (Pfizer Terramycin) and Oftalmotrofica vitaminized (Farve Laboratory).
As primary result, the mean duration of each disease, with or without the treatment under study were compared. Secondly, morbidity and mortality rates were studied for each of the diseases in both groups (the treated and the untreated) and the morbidity corresponding to the adverse effects of each treatment was communicated. Results were statistically compared through the use of the test for the comparison of median populations z ($n>400$ per group).
Here we present new evidence showing that pharmacologically induced histamine release significantly reduces the length and severity of three severe viral diseases in dogs.
The Oxitetracycline (terramycin) produces an intense local reaction, painful for 24 to 72 hours, but usually well tolerate.
The author, after many years, has considered histamine not only as endogenous antiviral but also as endogenous antibiotic.

HISTAMINE H_4 LIGAND DATABASE

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The H_4 ligand database was initiated and is being organised by Gabriella Coruzzi as part of COST Action BM0806 „Recent advances in histamine H_4R research". The database was designed to provide information on ligands used in various in vitro and in vivo test models. It is well known that inconsistencies have been reported concerning receptor binding and functional behaviour of the reference H_4R antagonist JNJ7777120 (and of other ligands). The effects of many H_4R ligands are highly species dependent. The database will help researchers in designing experimental assays with the most appropriate ligand and thus obtaining meaningful results and a better understanding of the role of H_4Rs in various tissues and species. The Database will summarise both published and unpublished data and input from any researcher working on H_4 is much appreciated. The aim is to evidence, in particular, intriguing data or data which apparently does not fit in the traditional pharmacological scheme and for this reason often remain unpublished and unknown to other researchers. The H_4 ligand database was one of the important deliverables of COST Action BM0806. Access to the database is free to any researcher interested who sends their contact details and is available as a shared spreadsheet on Googledocs. Expressions of interest should be sent to Astrid Sasse: sassea@tcd.ie who maintains the online version of the database which will be updated every few months and as appropriate.

THE HISTAMINE METHODS & TOOLS DATABASE - READY TO USE

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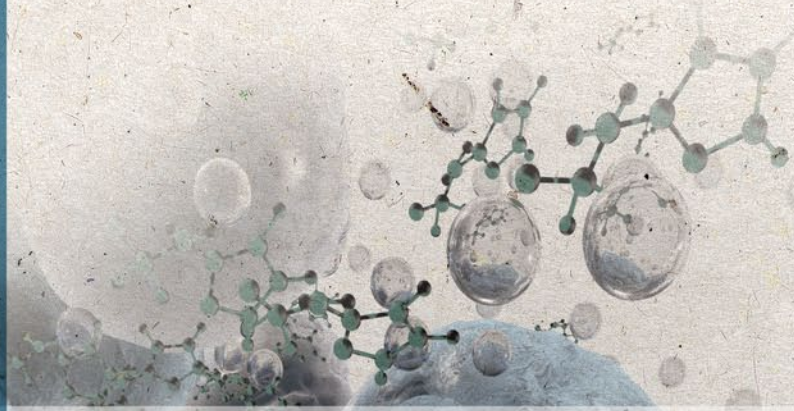
A major goal of COST Action BM0806 Recent Advances in Histamine Receptor H_4R Research was to establish and run an internet database covering all methods and tools available at participating institutions. The Histamine Methods & Tools Database (HMTD) is now online at www.i-med.ac.at/hmtd and is ready to use. The database is subdivided into the sections General Methods, Histamine Methods, Histamine Tools, In Vitro Models, Animal Models, and Patient Studies. Detailed instructions for submitting protocols can also be found on the website and everybody is very welcome to contribute to this collection and comment on its content. A number of protocols have been contributed already and it is expected that the collection will grow rapidly as more colleagues send their methods and description of tools useful for histamine and histamine receptor research. In its final version, the Histamine Methods & Tools Database will provide a comprehensive collection of all available methods with ready-to-use protocols, give contact information for method based inquiries, inform about sources of critical tools and will identify areas where new methods, techniques or tools should be developed.



DYNAMIC REGULATION OF WAKE-ACTIVE NEURONS

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The histaminergic and hypocretin neurotransmitter systems are essential components in the regulation of sleep-wake state. We studied the regulation of these wake-active neurons in larval and adult zebrafish (Danio rerio) by methods ranging from molecular biology and imaging to behavioral assays. We found that the adult histaminergic system in zebrafish consists of 41-45 neurons in both sexes. The system in zebrafish resembles that of mammals, as both GABA and galanin were coexpressed with histamine. Inhibition of histamine synthesis by transient knockdown of histidine decarboxylase (hdc) with morpholino oligonucleotides (MO) resulted in lack of histamine at the larval stage, and was associated with an impairment of sensorimotor function. The behavioral deficit was rescued by overexpression of hdc mRNA. The development of the hypocretin neurons was regulated in a histamine receptor subtype 1 (hrh1) dependent manner, as both hdc MO and the hrh1 antagonist pyrilamine treatment resulted in significantly fewer hypocretin mRNA positive neurons. The effect was rescued by overexpression of hdc mRNA. The development of the histaminergic neurons was studied in a novel presenilin1 mutant zebrafish line. We found that histaminergic neurons develop in a γ-secretase-dependent manner: at the larval stage the number of histaminergic neurons was significantly lower compared with the wild-type animals. At adult stage the histaminergic neuron number was significantly increased in the presenilin1 mutant fish compared with adult wild-type animals. The change in histaminergic neuron numbers was associated with changes in histamine driven behaviors. The newly generated histaminergic neurons may be a result of neurogenesis or neurotransmitter respecification. Taken together, these results offer new insight into the dynamic regulation of wake active neurons.

HISTAMINE MICROINJECTED INTO THE CEREBELLAR VERMIS IMPROVES MEMORY CONSOLIDATION OF INHIBITORY AVOIDANCE IN MICE

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Studies have revealed direct histaminergic projections from the tuberomamillary nucleus to the cerebellum, and our previous study showed that histamine microinjected into the cerebellar vermis impairs emotional memory consolidation in mice when using the Trial 1/2 protocol in an elevated plus-maze. This study investigated the effect of histamine microinjected in the cerebellar vermis on the memory retention of inhibitory avoidance learning in mice. The cerebellar vermis of male mice (Swiss Albino) were implanted with guide cannulae. After three days of recovery, the inhibitory avoidance test was performed. Immediately after a training session, animals received a microinjection of saline or histamine (0.54, 1.36, 2.72 or 4.07 nmol/0.1 microliter) in the cerebellar vermis. Twenty-four hours later, a retention test was performed to evaluate memory consolidation. The data were analyzed using one-way Analysis of Variance (ANOVA) and Duncan´s tests. ANOVA indicated differences between retention latency in mice injected with saline versus histamine. Post hoc analysis showed a significant increase in retention latency of animals that received histamine (1.36 nmol) in relation to the control group ($p<0.05$). In addition, there were differences between groups microinjected with 1.36 nmol histamine and with 2.72 nmol ($p<0.01$) and 4.07 nmol ($p=0.04$) histamine. These two higher doses showed a decrease in latency time in relation to 1.36 nmol histamine. These results indicated that 1.36 nmol histamine facilitates the memory retention of inhibitory avoidance in mice, suggesting a different role for histamine in a memory model that uses punishment.

EVIDENCE FOR ANALGESIC ACTION OF AESCULETIN IN CARRAGEENAN INDUCED INFLAMMATION

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Aesculetin is considered as a drug which might be used in treatment of osteoarthritis, rheumatoid arthritis, various types of cancer, leukemia.
Aesculetin is natural coumarin compound which possesses antioxidant, anti-tumor, anti-inflammatory, antiproliferative and neuroprotective properties. It is described as a potent 5-LOX inhibitor. Because presence of BLT1 receptor has been discovered on sensory neurons, it is suggested that LTB4 might act directly on primary afferent neurons reducing the nociceptive threshold. The main source of leukotrine B4 (LTB4) which mediates different inflammatory events and induce hypernociception are neutrophils. Histamine is mediator that plays important role in leukotriene B4 production and mast cell-dependent neutrophil recruitment. We decided to investigate whether aesculetin possess analgesic activity and analyze its action in acute inflammatory pain model in rats.
Inflammation was induced by injection of 1% carrageenan into the hindpaw of male Lewis rats weighting 250-350 g. The pain threshold was determined by using mechanical stimuli (the Randall-Selitto test) and thermal stimuli (Plantar test method and Tail-flick method). Pain threshold was assessed 30 minutes and 1 hour after intraperitoneall Aesculetin administration. After one hour animals were anesthetized and blood from heart was collected for morphological and biochemical analysis.
Our result presents for the first time that aesculetin posses analgesic activity in acute pain and acute inflammatory pain models in rats against mechanical and thermal stimuli.

EXPLORING TRANSCRIPTIONAL NETWORK CAUSALLY ASSOCIATED WITH POLLINOSIS BY TOLUENE-2,4-DIISOCYANATE-SENSITIZED RATS

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As allergy is a multifactorial disorder with abnormal up-regulation of the allergy-sensitive gene expression, drugs that decrease the expression levels of these genes should be good therapeutics. Recently, we demonstrated that the histamine H_1 receptor (H_1R) gene is an allergy-sensitive gene, i.e., the H_1R expression level affects the severity of allergy symptoms. We also found that the expression level of H_1R was strongly correlated with the expression levels of histidine decarboxylase (HDC) and IL-5 in patients with pollinosis, suggesting these genes are regulated by common signaling. We examined the effect of prophylactic treatment with antihistamines on TDI-induced nasal symptoms and up-regulation of H_1R, HDC, IL-4, and IL-5 gene expression in the nasal mucosa of toluene-2,4-diisocyanate (TDI)-sensitized rats.

Six-week-old male Brown Norway rats were sensitized and provocated with TDI. Nasal symptoms were scored for 10 min immediately after TDI-provocation. H_1R, HDC, IL-4, and IL-5 mRNA were determined by real-time quantitative RT-PCR. H_1R protein was determined by [^{3}H]mepyramine binding assay. HDC activity and histamine content were measured by HPLC with o-phthalaldehyde fluorometric detection system. IL-4 and IL-5 proteins were analyzed by ELISA.

Intranasal application of TDI causes nasal symptoms such by sneezing and watery rhinorrhea. Application of TDI also increased H_1R, HDC, IL-4, and IL-5 in both protein and mRNA. Pretreatment with antihistamines significantly reduced TDI-induced nasal symptoms and up-regulation of these genes. Intranasal application of histamine to non-TDI-treated rats up-regulated H_1R, HDC, IL-4, and IL-5 mRNA.

These data suggest that H_1R, HDC, IL-4, and IL-5 genes compose transcriptional network causally associated with pollinosis and the expression of these genes is regulated by histamine signaling. TDI-sensitized rats may be useful model to identify genes involved in this network and to find additional drug targets.

QUERCETIN INHIBITS TRANSCRIPTIONAL UP-REGULATION OF HISTAMINE H_1 RECEPTOR VIA SUPPRESSING PROTEIN KINASE C-D/ EXTRACELLULAR SIGNAL-REGULATED KINASE/POLY(ADP-RIBOSE) POLYMERASE-1 SIGNALING PATHWAY IN HeLa CELLS

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Histamine H_1 receptor (H_1R) gene is up-regulated in patients with allergic rhinitis and H_1R expression level strongly correlates with the severity of allergy symptoms. Accordingly compounds that suppress the H_1R gene expression are promising as useful anti-allergic medications. Recently, we demonstrated that histamine or phorbol-12-myristate-13-acetate (PMA) stimulation induced the up-regulation of H_1R gene expression through the protein kinase Cδ (PKCδ)/extracellular signal-regulated kinase /poly(ADP-ribose) polymerase-1 signaling pathway in HeLa cells expressing H_1R endogenously. Quercetin is one of the well-characterized flavonoids and it possesses many biological activities including anti-allergic activity. However, effect of quercetin on H_1R signaling is remained unknown.

We examined the effect of quercetin on stimuli-induced up-regulation of H_1R gene expression in HeLa cells. We also investigated its in vivo effects on the toluene-2,4-diisocyanate (TDI)-sensitized allergy model rats.

Six-week-old male Brown Norway rats were sensitized and provocated with TDI. Allergy-like symptoms were scored for 10 min immediately after TDI-provocation. H_1R and IL-4 mRNA were determined by real-time quantitative RT-PCR. Inositol phosphates accumulation assay was performed in myo-[^{3}H]inositol labeled HeLa cells. Phosphorylation of Tyr^{311} of PKCδ was analyzed by immunoblot analysis. Subcellular localization of PKCδ was immunocytochemically determined using confocal microscope.

Quercetin suppressed stimuli-induced up-regulation of H_1R gene expression. Quercetin also inhibited stimuli-induced phosphorylation of Tyr^{311} of PKCδ and translocation of PKCδ to the Golgi. Pretreatment with quercetin for 3 weeks suppressed TDI-induced nasal allergy-like symptoms and elevation of H_1R mRNA in the nasal mucosa of TDI-sensitized rats.

These data suggest that quercetin suppresses H_1R gene expression by the suppression of PKCδ activation through the inhibition of its translocation to the Golgi.

THE HUMAN HISTAMINEGIC SYSTEM IN HEALTH AND NEUROPSYCHIATRIC DISORDERS BRAIN: A POSTMORTEM STUDY

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The hypothalamic tuberomamillary nucleus (TMN) is the exclusive source of neuronal histamine. The histaminergic system is crucially involved in a number of basic physiological brain functions. We performed quantitative radioactive in situ hybridization of histidine decarboxylase (HDC), the rate limiting enzyme for histamine production, to study changes in histamine production. Moreover, expression changes of the histaminergic receptors and of the inactivating enzyme histamine methyltransferase (HMT) were studied by qPCR in specific projection areas in these disorders.

A diurnal fluctuation in HDC-mRNA expression was found for the first time in controls while a disorder of this diurnal fluctuation was observed in neurodegenerative diseases including Parkinson's (PD), Huntington's (HD) or Alzheimer's (AD) disease.

In PD there was an abundant presence of Lewy bodies and Lewy neurites in the TMN, which were presumed to indicate a strong degeneration of this nucleus. In spite of that, HDC-mRNA in the TMN remained unaffected in either early or late stage of PD. A higher histaminergic receptor expression and HMT was found in the substantia nigra and putamen, which may play a protective role against augmentation of histamine in these areas of PD.

HD results from an abnormal polyglutamine extension in the N-terminal region of the huntingtin protein. In the hypothalamus, the aggregation of this protein was more strongly in the TMN. Moreover, we found that the HDC-mRNA levels were increased as was the HMT expression in other brain areas.

In AD, HDC-mRNA levels in the TMN were unchanged, in spite of TMN neuronal loss. In the course of AD only females had increased prefrontal cortex expression of H_3R- and HMT-mRNA.

In addition, alterations of histaminergic system in depression or schizophrenia will also be discussed. Based upon our postmortem findings, potential clinical implications will be mentioned.

EHRS International Anthem

Mel. Polly Waddle Doodle

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

1. We talk of toxicosis / migraine, shock or halitosis
Singing Histaminosis all the day.
Trauma, burns and inflammation / headache, pain and constipation
Singing Histaminosis all the day.

2. You give asthmatic wheezes / the allergic sneezes
Singing Histaminosis all the day.
Though obscure as yet, the fact is / you´re involved in anaphylaxis
Singing Histaminosis all the day.

3. Since the time of Dale and Barger / your files are longer, larger
Singing Histaminosis all the day.
The control of circulation / then gastric stimulation
Singing Histaminosis all the day.

CHORUS

4. Mast cells by the dozen / and basophils your cousin
Singing Histaminosis all the day.
They come and they go / fluctuate to and fro,
Singing Histaminosis all the day.

5. We heard a lot of groaning / from the upstart, Serotonin,
Singing Histaminosis all the day.
Down with 5-hydroxytrypta / and up with good old hista,
Singing Histaminosis all the day.

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

CHORUS

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

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

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

CHORUS

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

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

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

CHORUS

13. And in nineteen eighty four / back in Florence like before,
Singing Histaminosis all the day.

Then in Aachen eighty five / Charlemagne became alive,
Singing Histaminosis all the day.

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

15. G.B. West was then cheered / for the ten years we´d been steered,
Singing Histaminosis all the day.
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.

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

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Publication of the EHRS meeting's proceedings

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

Abstract Instructions

Please arrange the abstract as follows:

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