European Histamine Research Society

50th Annual Meeting

1st - 3rd September 2022

Established 1971

Leibnizhaus

HANNOVER, Germany

PROGRAMME AND ABSTRACT BOOK

1 Congress Venue Leibnizhaus

2 Hotel Am Leineschloss

3 Gala Dinner Gartensaal Neues Rathaus

Welcome Message

It is a great pleasure and honor for us to welcome you in Hannover to join the 50th Anniversary EHRS Meeting. It took more than 2 years we had to wait to welcome you at this face-to-face conference due to the COVID pandemic. Like the 49 preceding EHRS conferences the meeting shall bring leading international scientists from academia, clinical medicine and industry together serving as a forum for the worldwide exchange of novel research results and ideas as well as providing good opportunities for new cooperations in the field. We guess that you may be as excited as we are to meet scientific colleagues and friends in person again after hundreds of online conferences. The 50th Anniversary EHRS Meeting was organized together with the Germany Society of Allergology and Clinical Immunology (DGAKI), which helped us with its local infrastructure.

Did you know? In 1981 the 10th EHRS Meeting was already hosted in Hannover, organized by a local committee under the lead of Professor Sewing, Head of the Dept Pharmacology of Hannover Medical School. We highly recommend to read the minutes of this congress with a number of interesting congress contributions, some of them very entertaining (https://www.ehrs.org.uk/wp-content/uploads/2016/10/1981-Meeting-Report.pdf). Before and after that event in 1981 histamine research has been focused by different working groups in three universities of this city: Hannover Medical School (the only independent public Medical University in Germany), Hannover Veterinary University and the Leibniz University with various faculties in life sciences.

Hannover is the capital of Lower Saxony and considered as one of Germany´s greenest cities: The city forest Eilenriede is the largest forest in Europe within city limits. Moreover, you can visit parks, the shores of the city lake Maschsee and the famous Royal Gardens of Herrenhausen which offer attractive recreation areas for citizens and visitors. Beyond its high recreational values Hannover has earned an international reputation as a global trade fair and congress location.

Our conference will take place in the Leibnizhaus, located in the center of the historic old town of Hannover. The Leibnizhaus serves a central guesthouse for academic guests visiting the universities, and as venue for numerous scientific events initiated or organized by members of Hannover´s universities. The original, historic Leibnizhaus was built in 1499 as the residence of a patrician family and was located about a 5 minutes‘ walk apart from our current guest and conference center at the Schmiedestrasse.

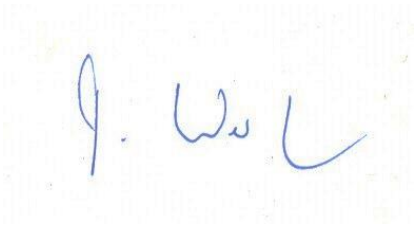
The Leibnizhaus got its name in the 19th century in memory of one of the most famous residents of Hannover: the philosopher and polymath Gottfried Wilhelm Leibniz. Leibniz moved to Hannover in 1698 and lived at the Schmiedestrasse 10 until his death in 1716. Here he developed

Welcome Message

his essential philosophical and mathematical concepts. He inspired history and religion and gave impetus to law and the general organization of science. We hope that his creative overarching scientific spirit might also inspire your research in the future and helps that the complex biology of histamine will be better understood!

We wish you a rewarding personal meeting and a pleasant intriguing stay in Hannover.

On behalf of the Organizing Committee



Thomas Werfel; Hannover Medical School, Germany

Holger Stark; Heinrich Heine University, Düsseldorf, Germany

Previous Meetings

1970's

1971 Lodz
1972 Paris
1973 Marburg
1974 Copenhagen
1975 Florence
1976 Paris
1977 London
1978 Lodz
1979 Stockholm

1980's

1980 Visegard
1981 Hannover
1982 Bled
1983 Brighton
1984 Florence
1985 Aachen
1986 Odense
1987 Strbske Pleso
1988 Copenhagen
1989 Breda

1990's

1990 Kuopio
1991 Marburg
1992 Malaga
1993 Cologne
1994 Budapest
1995 Moscow
1996 Antwerp
1997 Seville
1998 Lodz
1999 Lyon

2000's

2000 Nemi (Rome)
2001 Turku
2002 Eger
2003 Noordwijkerhout
2004 Bergisch-Gladbach
2005 Bled
2006 Delphi
2007 Florence
2008 Stockholm
2009 Fulda

2010's

2010 Durham
2011 Sochi
2012 Belfast
2013 Lodz
2014 Lyon
2015 Malaga
2016 Florence
2017 Amsterdam
2018 Dublin
2019 Krakow

General Information

Venue

The conference will take place in the newly built **Leibnizhaus,** located in the center of the historic old town of Hannover, Holzmarkt 5, 30159 Hannover.

The **Holzmarkt** is dominated by the faithfully reconstructed House of the philosopher and polymath **Gottfried Wilhelm Leibniz (1646-1716)**, who served at the court in Hannover for 40 years. Behind the Renaissance facade of the house there are the modern conference rooms where our 50th Annual EHRS Meeting takes place. In front of the **Leibnizhaus** you find the **"wishing well"** (please turn the wheel) designed by **Oskar Winter**.

General Information

Travel Information

Reaching Leibnizhaus from the airport: The S-Bahn S5 will take you to the Central Station in 25 minutes.

Reaching Leibnizhaus from the Central Station: Take subway number 3, 7, or 9 (in the direction of Wettbergen, Empelde) to the second stop (‘Markthalle/ Landtag’). Leave the subway station at the exit towards ‘Marktkirche’. Pass the church and then turn left into ‘Kramerstraße’. At the end of the street, you will find the ‘Holzmarkt’, the fountain and the historical facade of the Leibnizhaus.

Registration Desk Opening hours

The registration desk is located in the venue, just next to the entrance.

Thursday 08:30 – 18:00 h

Friday 08:30 – 17:00 h

Saturday 08:30 – 14:00 h

WiFi Guest Network Leibnizhaus Hannover

Wifi: (SSID): UHEvent

Password: E4fJL9Yc

Homepage: www.uni-hannover.de/leibnizhaus

Tel. +49 511 762-4450

Fax. +49 511 762-4460

E-Mail: Leibnizhaus@zuv.uni-hannover.de

General Information

Oral Communications

Oral presentations are scheduled not to extend 15 min, which includes discussion.

Please ensure that the file with your presentation (pdf or Microsoft PowerPoint format) is handed over at the registration desk on the morning of your presentation at the latest. Alternatively, you may email your presentation in advance to Neumann.Detlef@mh-hannover.de

The file of your presentation should be named as follows:

'SessionNumber_Presenting Author.pptx', e.g., '**O1_Mustermann.pptx'.**

Poster Presentations

An individual number will be assigned to each single poster.

Posters should be in A0 format, portrait oriented (height 120 cm x width 90 cm, no landscape). **The title** should be typed in capital letters and the authors should appear next, underlining the author who presents the poster, followed by the address of the authors.

Logos should not exceed the final size of 5×5 cm and should be placed in the upper left corner of the poster only. Poster number should be placed in upper right corner.

All posters will also be presented in a guided poster walk. Each presenting author will be given up to **five minutes** to make a brief oral presentation introducing their poster.

In all abstracts printed in this book for oral communications or for poster presentations, only the **institution of the presenting author** is given, as requested in the abstract instructions.

The Poster Jury will review all posters and ask questions at 2 poster sessions:

- session I September, 1; 14.00 – 15:30 h *(posters **1**- **7**)*
- session II September, 2; 14.15 – 15:30 h (*posters **8-15)***

Poster Prizes will be announced at the Gala Dinner.

COMMITTEES & PANELS

Local Organization Team

Thomas Werfel, Hannover Medical School, Hannover, Germany
Detlef Neumann, Hannover Medical School, Hannover, Germany
Holger Stark, Heinrich-Heine University, Düsseldorf, Germany
Susanne Mommert, Hannover Medical School, Hannover, Germany
Wiebke Filsinger, Hannover Medical School, Hannover, Germany

We would like to thank Pia Dumke, Hannover Medical School, Hannover, Germany for kindly providing her photos of Hannover to illustrate this Abstract Book.

Abstract Evaluation & Student Bursary Award Committee

Bernhard Gibbs, Carl von Ossietzky University of Oldenburg, Oldenburg, Germany
Misunobu Mio, Shujitsu University, Okayama, Japan
Susanne Mommert, Hannover Medical School, Hannover, Germany
Detlef Neumann, Hannover Medical School, Hannover, Germany

Poster Award Panel

Wieslawa Agnieszka Fogel, Medical University of Lodz, Lodz, Poland
Ilona Obara, University Newcastle, Newcastle UK
Susanne Mommert, Hannover Medical School, Hannover, Germany
Katrin Schaper-Gerhardt, Hannover Medical School, Hannover, Germany

Social Programme

Guided City Tour

On Thursday September 1, 2022 at 18:30 h we have **a guided City Tour** (90 minutes of walking) through the very beautiful small **old town and other places of Hannover**.

Interestingly, the houses with half timbered facades from the 16th and 17th centuries were spread all over the city. After the World War II they were taken from throughout the city and collected in one place.

Of the medieval city towers, only the **Beginenturm** at the historical museum still exists.

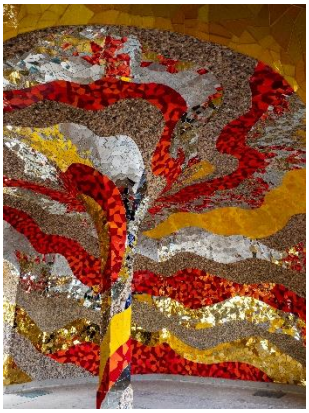
The first town hall and the oldest secular building of the city was the **Old Town Hall,** which is located on the **Market Place** together with the **Market Church.** Both form the southernmost group of buildings of the **Northern Brick Gothic style.** The Evangelical Lutheran **Market Church of St. Georgii et Jacobi** is the oldest of the three parish churches in Town. With its 97 meter-high tower the church is one of Hannover´s landmarks.

The river **Leine** flows between the old town and the quarter **Calenberger Neustadt**. On the banks of the river is the **Leineschloss,** today the seat of the **Lower Saxony Parliament** and formerly a royal residence, dates back to a Franciscan monastery founded in the 12th century, which was abolished in 1533. After the Duke of Calenberg chose Hannover as his residence in 1636, the north-western wing of the palace was renovated in 1742. The remaining parts go back to designs by the **master builder Georg Friedrich Laves**, who was very active in Hannover and completely rebuilt the house between 1816 and 1844. After being destroyed in the World War II, the **Leineschloss** was rebuilt between 1956 and 1962 and the south wing was replaced by a modern plenary hall extension.

Social Programme

Another Hannover landmark has stood on the other bank of the river Leine since 1974: **the Nanas by Niki de Saint Phalle.** Beyond **the Nanas** due to the special relationship of Hannover to the late French artist, Niki de Saint Phalle had other works in public spots in Hannover like "**The Cave"** in the **Great Garden Herrenhausen** or in the **Sprengel Museum Hannover**, a museum of modern art and, with a focus on German Expressionism and French Modernism. The underground shopping passage from the **Central Station** to **Kröpke** was named **"Niki de Saint Phalle Promenade**".





Walking through Kreuzstraße you come to **Ballhofplatz**. Built in 1649-64, the **Ballhof** was originally a sports hall where the court society could play badminton in all weathers. Later it became a meeting hall and is finally used today as a theater for the **Junges Schauspiel** Hannover. The **Ballhof** is one of Hannover's most beautiful secular building.

Outside the historic city, also representing one of Hannover's landmarks that should not be missed on any visit to Hannover, is the magnificent building of the **New Town Hall.** The **New Town Hall** was completed in 1913 and represents a mix of different architectural styles, especially Neo-Renaissance and Baroque. Of interest: Because the ground was so marshy,

the foundations was built on more than 6000 piles! The **New Town Hall** is the **City Hall** of the Lower Saxony capital and headquarter of the Hannover City administration under its Lord Mayor.

The building is embedded in the **Maschpark** with its small lake the **Maschteich**. The dome of the **New Town Hall**, with its observation platform is reached by the domes lift, which is unique in Europe, with its arched course.

The view from the top widens over the **Maschpark** to the **Maschsee,** an artificial lake made by man, which is the largest body of water in Hannover and allows numerous watersports. When visibility is good the **Harz Mountain** can be seen.

Highlights in front of the **New Town Hall:** the bronze statue of the archer aiming directly at the Lord Mayor's seat, the **Trammplatz** named after the former **City Director Heinrich Tramm,** with its large-mouthed flower pattern, and the **Klaus Bahlsen Fountain** by artist Ludger Gerdes.

Also located on the edge of the **Maschpark** is the **Museum Lower Saxony State**. It houses five collections presented in three worlds. **Huber Stier** designed the representative museum building in Neo-Renaissance style. After four years of construction, the building was completed in 1902.

On the eastern edge of the old town on a former windmill the **Opera House** was built by the court architect **Georg Ludwig Friedrich Laves** as the Royal Court Theater in the years 1845-1852 from Wealden sandstone. Now the Hannover Opera House is the venue for opera, ballet and concerts by the Lower Saxony State in Hannover. The orchestra of the Opera House is the Hannover State Orchestra of Lower Saxony.

Social Programme

In the western part of the city are located the **Herrenhausen Gardens** consisting of the **Great Garden**, the **Berggarden**, the **Georgengarden** and the **Welfengarden.**

The **Great Garden** in Herrenhausen is one of the most important baroque gardens in Europe and is the historical centerpiece of the Herrenhausen Gardens. The starting point for the **Great Garden** in Hannover was the **Herrenhausen Palace,** originally a baroque building from the 17th century. It was redesigned in the 19th century in the spirit of classicism, but the main building was destroyed during the World War II. Ten years ago, the classical castle was reconstructed with the financial help of the Volkswagen Foundation.

The **Berggarden**, also located in Herrenhausen, developed from a vegetable and cultivation garden into a botanical garden.

Social Programme

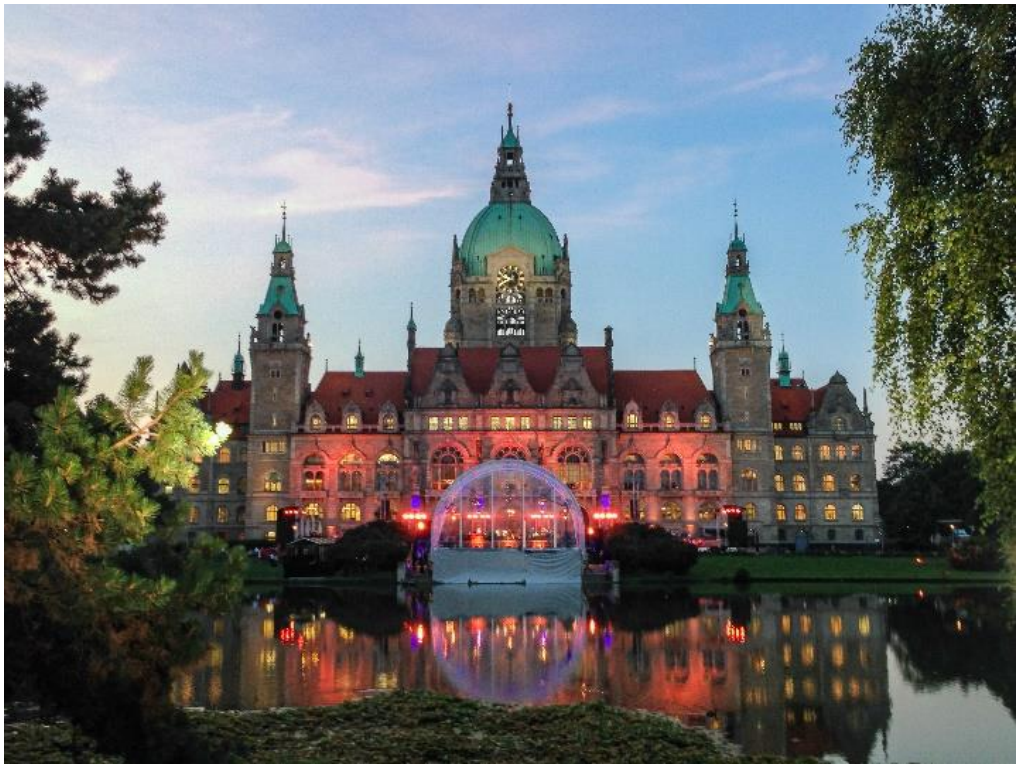
The **Georgengarden** is located to the east of the Great Garden and, like the **Welfengarden**, belongs to the Nordstadt district. Both gardens are laid out in the style of English landscape gardens and are freely accessible.

Embedded in **Welfengarden** is the **Gottfried Wilhelm Leibniz University Hannover (LUH)** representing the largest University in Lower Saxony. **The LUH** is housed in the **Welfen Castle**, which was built from 1857 to 1866 according to constructions by the architect **Christian Heinrich Tramm**. The castle was formerly planned as a summer residence for the royal family.

Gala Dinner

On Friday September 3, 2022 at 19:30 h we have our traditional Gala Dinner in the **Gardensaal,** which is located on the lower floor of the **New Town Hall.** When the weather is nice, we can stay outside at the beginning and enjoy the view over the beautiful Maschpark.

Bistro "Der Gartensaal" | Historische Bauten | Architektur & Geschichte | Kultur & Freizeit (hannover.de)



Invited Speakers

GB West Lecture

Prof. Wolfgang Bäumer, Freie Universität (FU) Berlin, Berlin Germany

Title: *Histamine in atopic dermatitis, particularly animal models of atopic dermatitis*

Prof. Thomas Werfel, Hannover, Medical School, Hannover, Germany

Title: *Novel treatments in atopic dermatitis – beyond histamine*

Prof. Wieslawa Agnieszka Fogel, Medical University of Łódź, Łódź, Poland

Title*:* *Multitarget drugs directed towards histamine and biogenic amines in neurodegenerative diseases*

Prof. Rob Leurs, Vrije Universiteit (VU) Amsterdam, The Netherlands

Title: *Chemical biology tools for the histamine receptor family*

Prof. Detlef Neumann, Hannover, Medical School, Hannover, Germany

Titel: *Histamine in intestinal diseases*

Programme

Thursday September 1, 2022

09:00 – 11:00	**Registration & poster mounting**
	EHRS Council Meeting
11:00 – 17:00	Chairs: Vanina A. Medina, Pontifical Catholic University of Buenos Aires, Buenos Aires, Argentina Katerina Tiligada, National and Kapodistrian University of Athens, Athens, Greece
11:00 – 11:45	**GB West Lecture**
	Prof. Wolfgang Bäumer, Freie Universität Berlin, Berlin, Germany
GB	*Histamine in atopic dermatitis, particularly animal models of atopic dermatitis*
11:45 – 12:15	**Coffee break**
12:15 – 13:00	**Clinical Lecture I**
	Prof. Thomas Werfel, Hannover Medical School, Hannover, Germany
L1	*Novel treatments in atopic dermatitis – beyond histamine*
13:00 – 14:00	**Lunch Break**
14:00 – 15:30	**Poster viewing/presentation I**
15:30 – 17:00	**Scientific Lecture I**
	Histamine in Allergy and Inflammation
15:30 – 15:45	S. Marri, University of Florence, Florence, Italy
O1	*Role of adenosine A3 receptors (A3AR) in bleomycin-induced lung fibrosis in mice*

Programme

15:45 – 16:00	E.J. Wunschel, Hannover Medical School, Hannover, Germany
O2	*Investigating the interplay of TRP channels and histamine receptors in a human neuron-keratinocyte co-culture model*
16:00 – 16:15	L. Beyer, Hannover Medical School, Hannover, Germany
O3	*Histamine increases the expression of IL-18 and the IL-18R on human eosinophils from AD patients*
16:15 – 16:30	J.P.S. Fernandes, Universidade Federal de São Paulo, São Paulo, Brazil
O4	*Anti-inflammatory effects of LINS01007, a histamine H_4R antagonist, in mice DSS-induced colitis model*
16:30 – 16:45	J. Vidak, Freie Universität Berlin, Berlin, Germany
O5	*Role of histamine H4 receptor in chronic dermatitis - results with the H4 receptor knockout mouse*
16:45 – 17:00	K. Popiolek-Barczyk, Polish Academy of Sciences, Krakow, Poland
O6	*A novel antagonist of histamine H_4 receptor, JSJ, as a potential candidate for the treatment of neuropathic pain – in vivo and in vitro studies*
17:00 – 17:30	**Coffee break**
17:30 – 18:30	**Opening Ceremony** Welcome Prof. Katerina Tiligada, President EHRS Welcome Prof. Thomas Werfel, Prof. Holger Stark Presentation of the bursary winners by Prof. Katerina Tiligada
18:30	**City Tour through Hannover** Meeting point: Beginenturm, Pferdestrasse Endpoint: Ballhof Duration: ca. 1,5 h
20:00	**Come Together: Welcome Drinks and Food in the Leibnizhaus**

Programme

Friday September 2, 2022

09:00 – 10:30 **Scientific Lecture II**

Immunomodulation by Histamine

Chairs:

Madeleine Ennis, Queen's University Belfast, Belfast, United Kingdom

Silvia Sgambellone**,** University of Florence, Florence, Italy

09:00 – 09:15 M. Raithel, Malteser Waldkrankenhaus St. Marien, Erlangen, Germany

O7 *Role of mast cells in gastroenterology and endoscopy*

09:15 – 09:30 B. P. Ganesh, University of Texas Health Science Center, Houston, USA

O8 *Stabilizing histamine release in mast cells signaling from the gut mitigates neuroinflammation in the brain post stroke*

09:30 – 09:45 S. Mommert, Hannover Medical School, Hannover, Germany

O9 *Th2 cytokines and histamine regulate the expression of enzymes in the biosynthesis of leukotrienes, release of cysteinyl leukotrienes and their receptor expression in human mast cells*

09:45 – 10:00 S. Lietzau, Hannover Medical School, Hannover, Germany

O10 *Expression and regulation of the cysteinyl leukotriene system in human monocytes and macrophages by cytokines playing a role in atopic dermatitis*

10:00 – 10:15 V.A. Medina, Pontifical Catholic University of Buenos Aires, Buenos Aires, Argentina

O11 *Histamine H_4 receptor is expressed in human T-cell lymphoma and its activation induces antitumor effects*

Programme

10:15 – 10:30 R. Khanferyan, Peoples` Friendship University of Russia, Moscow, Russian Federation

O12 *Histamine and Histamine H_3 receptors are involved in the synthesis of IL-17 in psoriasis.*

10:30 – 11:00 **Coffee break**

11:00 – 13:15 Chairs:
Pertti Panula, University of Helsinki, Helsinki, Finland
Bassem Sadek, United Arab Emirates University, United Arab Emirates

11:00 – 11:45 **Invited Lecture II**
Prof. Wieslawa Agnieszka Fogel, Medical University of Łódź, Łódź, Poland

L2 *Multitarget drugs directed towards histamine and biogenic amines in neurodegenerative diseases*

11:45 – 13:15 **Scientific Lecture III**
Histamine and Nervous Function

11:45 – 12:00 X. Ligneau, Bioprojet-Biotech, Saint-Grégoire, France

O13 *Histamine H3 receptor inverse agonists/antagonists: new therapeutic agents for the treatment of excessive daytime sleepiness in patients with obstructive sleep apnoea*

12:00 – 12:15 I. Obara, Newcastle University, Newcastle upon Tyne, UK

O14 *Selective and peripherally acting histamine H_3 receptor (H_3R) antagonist PF-0868087 produces analgesic effect in neuropathic pain via the mammalian target of rapamycin complex 1 (mTORC1) pathway in mice*

12:15 – 12:30 *H.L. Haas,* Heinrich-Heine-University, Düsseldorf, Germany

O15 *A brief history of histamine neurophysiology*

Programme

12:30 – 12:45 Ling Shan, Leiden University Medical Centre, Leiden, The Netherlands
O16 *Reduced numbers of corticotropin-releasing hormone neurons in narcolepsy type 1*

12:45 – 13:00 N, Eissa, College of Medicine & Health Sciences, United Arab Emirates University, United Arab Emirates
O17 *ST-2223, a novel multiple active H3R/D2R/D3R antagonist modulates brain neurotransmitters and enhance social interaction in BTBR mouse model of autism*

13:00 – 13:15 *O.A. Sergeeva,* Heinrich-Heine-University, Düsseldorf, Germany
O18 *OLHA modulates activity of mouse brain histaminergic neurons*

13:15 – 14:15 **Lunch break**

14:15 – 15:30 **Poster viewing/presentation II**

15:30 – 16:00 **Coffee break**

16:00 – 18:15 Chairs:
Holger Stark, Heinrich-Heine University, Düsseldorf, Germany
CongYu Jin, University of Helsinki, Helsinki, Finland

16:00 – 16:45 **Invited Lecture III**
Prof. Rob Leurs, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
L3 *Chemical biology tools for the histamine receptor family*

Programme

16:45 – 18:15	**Scientific Lecture IV**
	Molecular Pharmacology I
16:45 – 17:00	H. Jacob, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
O19	*Lysergic acid diethylamide is a full agonist at human cardiac H_2-histamine receptors*
17:00 – 17:15	C.M.S.Q. Aranha, Universidade *de* Federal de São Paulo, São Paulo, Brazil
O20	*Assessment of multitargeting compounds for histamine/ dopamine receptor subtypes and cholinesterases*
17:15 – 17:30	P.L. Chazot, Durham University, Durham, UK
O21	*Pharmacological rationale for use of a combination of Piracetam and Cinnarizine (Nootroparizine) for post-concussion syndrome: a case study*
17:30 -17:45	V.A. Medina, Pontifical Catholic University of Buenos Aires, Buenos Aires, Argentina
O22	*Nanomicellar formulation of Paclitaxel and histamine as a potential breast cancer chemotherapeutic system*
17:45 -18:00	*L.M Rayo-Abella,* Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
O23	*A novel transgenic mouse to study cardiac effects of histamine H_1 receptors*

Programme

18:00 – 18:15	W. Andrade, University of Regensburg, Regensburg, Germany
O24	*Molecular modifications on basic group and aromatic moiety revealed important information of structure-affinity relationship for LINS01 compounds*
19:30	**Gala Dinner, Gartensaal Neues Rathaus**

Programme

Saturday September 3, 2022

10:00 – 12:45 Chairs:

Joao Paulo Fernandes, Universidade Federal de São Paulo, Brazil

Joachim Neumann, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

10:00 – 10:45 **Invited Lecture IV**

Prof. Detlef Neumann, Hannover Medical School, Hannover, Germany

L4 *Histamine in intestinal diseases*

10:45 – 11:15 **Coffee break**

11:15 – 12:45 **Scientific Lecture V**

Molecular Pharmacology II

11:15 – 11:30 V.A. Medina, Pontifical Catholic University of Buenos Aires, Buenos Aires, Argentina

O25 *Antitumoral properties of novel histamine H_3 receptor antagonists for breast cancer treatment*

11:30 – 11:45 I.Josimovic, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

O26 *Pharmacological characterization of H_1R and H_3R small molecule photoligands – reaching beyond the UV spectrum*

11:45 – 12:00 S. Sgambellone**,** University of Florence, Florence, Italy

O27 *A histamine H_3 receptor (H_3R) antagonist-nitric oxide (NO) donor hybrid compound in the prevention of post-ischemic photoreceptor degeneration*

Programme

12:00 – 12:15	M. Dubiel, Heinrich Heine University, Düsseldorf, Germany
O28	*Histamine H_3 receptor antagonists influencing Aβ oligomerisation*
12:15 – 12:30	CongYu Jin, University of Helsinki, Helsinki, Finland
O29	*Detection of H3 and H4 receptor mRNA expression at the cellular level*
12:30 – 12:45	M. Gao, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
O30	*Pharmacological characterization of photocaged histamine H_3 and H_4 receptor agonists*
12:45 – 13:30	**Lunch break**
13:30 – 13:45	Closing the Meeting
13:45 – 15:00	**Honorary EHRS Membership Ceremony; Oration Rado Nosal** **EHRS General Assembly incl. elections**

Invited Lectures Abstracts

EHRS 2022 Hannover

GB West Lecture

GB

HISTAMINE IN ATOPIC DERMATITIS, PARTICULARLY ANIMAL MODELS OF ATOPIC DERMATITIS

Wolfgang Bäumer

Atopic dermatitis (AD) is a chronic relapsing pruritic inflammatory skin condition. AD is commonly associated with elevated levels of immunoglobulin E (IgE) often directed against environmental allergens. Histamine is a pro-inflammatory mediator that contributes particularly to a Th2 driven inflammatory reaction and acts directly as a pruritogen on sensory neurons mainly via activation of histamine H1 and H4 receptors. Unfortunately, there are not many studies that report the concentration of histamine in skin and/or serum of atopic patients or in animal models of AD. Nonetheless, in most of the determinations, elevated levels of histamine have been found under inflammatory conditions. We measured histamine via microdialysis in a canine model of AD and observed a transient increase of histamine directly after challenge with the relevant allergen (house dust mite antigen) but no elevation was seen e.g. 6 h or 24 h after challenge. In recent years, studies in histidine decarboxylase (HDC) knockout mice as well as with specific histamine H1 and H4 receptor antagonists helped us to elucidate the role of histamine in chronic allergic skin conditions. Particularly in chronic models of allergic dermatitis, general lack of histamine (HDC knockout mice) as well H4 receptor and to a lesser extend H1 receptor antagonists show a distinct reduction of both inflammatory and pruritic conditions. This was confirmed in a chronic ovalbumine induced dermatitis model in H4 receptor knockout mice. Interestingly, the strongest inhibition was observed by combined antagonism of both H1 and H4 receptors across varying mouse models of atopic dermatitis. These findings suggest that the dual inhibition of both H1 and H4 receptors might be the most promising strategy to address chronic AD related itch and inflammatory processes.

Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Germany

Wolfgang.baeumer@fu-berlin.de

Clinical Lecture

L1

NOVEL TREATMENTS IN ATOPIC DERMATITIS-BEYOND HISTAMINE

Thomas Werfel

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases in humans leading to a substancial loss of quality of life mainly. This is mainly caused by its intense pruritus associated with sleep disturbances and stigmatisation by eczema in the face and other visible areas of the skin. AD is considered to be mainly a T-cell driven skin disease but increases of other cell populations contributing to TH2 driven skin inflammation are known (e.g. mast cells, macrophages, dendritic cells). This leads to complex inflammatory interaction between cells and their mediators in the skin including histamine.

As discussed during the GB West lecture by Wolfgang Bäumer at this conference histamine (secreted by mast cells but also by basophils in the acute phase and by other myeloid cells in the skin) and its receptors have been described in animal models to play a role in AD both in mediating inflammation and pruritus in the skin. In humans there have been few clinical studies on effects of H1 or H2 receptor blockers in AD with more or less disappointing results - but some of them may have been underpowered by patient numbers. Both positive and negative results came from human studies with H4 receptor antagonists during the last years, and currently a large international study is still recruiting patients to study the effect of a novel H4 receptor blocker.

Classical approaches treating AD with corticosteroids and calcineurin inhibitors act very broadly on inflammatory cells. In 2017, the first Th2 directed antibody named dupilumab binding to the IL-4R alpha chain and thus inhibiting IL-4 and IL-13 was approved for the treatment of moderate to severe AD in humans. Last year the IL-13 specific antibody tralokinumab became available. The Janus kinase (JAK) inhibitors baricitinib, upadacitinib and recently abrocitinib inhibiting cytokine signals (induced by IL-4, IL-13, IL-31 and others) in the intracellular parts of their receptors and acting faster than the antibodies on inflammation and pruritus were approved for systemic treatment of AD between 2020 and 2022. With ruxolitinib and delgocitinib topical applicable JAK inhibitors became available in the USA resp.Japan but not yet in Europe.

Substances addressing other targets with impact on pruritus and inflammation, such as TSLP, IL-31, IL-22 or IL-33 -some of them being upregulated by histamine- have also been published with promising results on symptoms and signs of atopic dermatitis but are not approved so far.

To better understand the possible clinical impact of histamine in AD and to develop new integrated concepts it is useful for researchers and clinicians in the field to have an overview of novel therapeutical approaches. Moreover, AD may also serve as a model for other allergic/ atopic diseases in that context.

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Invited Lectures

L2

MULTITARGET DRUGS DIRECTED TOWARDS HISTAMINE AND BIOGENIC AMINES IN NEURODEGENERATIVE DISEASES

WA Fogel

Neurodegenerative diseases impose a heavy burden not only on individuals but also on world economies. Currently, according to data from the WHO, more than 55 million people live with dementia worldwide, and there are nearly 10 million new cases every year. Sixty to seventy percent are related to Alzheimer's disease (AD). Due to the general aging of the population, the number with AD is expected to rise to 78 million by 2030. The second leading neurodegenerative disease is Parkinson's disease (PD) affecting more than 10 million patients and their number is expected to double by 2030. Common for neurodegeneration is the loss of neurons resulting in a deficit in neurotransmitter levels and the formation of protein aggregates like beta-amyloid or alpha-synuclein deposits. Progressive neurotransmitter decrease concerns not only acetylcholine and dopamine, but also others (noradrenaline, serotonin, histamine) as reflected in symptoms. Thus, besides the cognitive and motoric problems, lack of motivation, and dysautonomia, the patients suffer from emotional, sensory, and sleep disturbances. As yet, the neurodegeneration remains incurable. The current treatment allows only for slowing the disease progression. To move away from polypharmacology with a risk of drug-to-drug interactions the rule of one drug for one target was recently replaced by a multitarget drug (MTD). Since the presynaptic histamine H3 receptor acts as auto- and heteroreceptor, controlling the release of histamine and acetylcholine, dopamine, noradrenaline, serotonin, glutamate, GABA, and some neuropeptides, this makes histamine H3 receptor antagonist/inverse agonist the excellent core drug. Preclinical data obtained for the drug that combines H3R pharmacophore with an inhibitory moiety directed towards a degradative enzyme e.g. acetyl/ butyrylcholinesterase and/or monoamine oxidase, or Aβ oligomerisation, or D2/D3R antagonist look promising as will be shown in this presentation.

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Invited Lectures

L3

CHEMICAL BIOLOGY TOOLS FOR THE HISTAMINE RECEPTOR FAMILY

Rob Leurs

The histamine research community has in the last decade been very active and generated a number of exciting new chemical biology tool for the study of histamine receptors, their ligands and their pharmacology. In this talk, I will describe the emergence of histamine receptor structural biology, the use of new receptor biosensors and the development of new ligands for fluorescent labelling or photopharmacological approaches (photocaging and photoswitching). All these new tools allow the research community to develop new approaches to study histamine receptors and their roles in (patho)physiology.

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Invited Lectures

L4

HISTAMINE IN INTESTINAL DISEASES

Detlef Neumann

Inflammatory bowel diseases (IBD), including its major manifestations ulcerative colitis and Crohn's disease, are recurring or continuous chronic inflammatory diseases of still unknown cause. IBD patients bear an increased lifetime risk of developing colitis-associated colorectal cancer (CAC). In healthy human colon tissue, at least H_1R, H_2R, and H_4R can be identified and enhanced concentrations of histamine (HA) are detected in affected tissues of patients suffering from colitis and/or CAC as compared to normal colon tissue, indicating a possible role for HA in these pathogeneses. Indeed, the involvement of HA and H_4R in animal models for colitis has already been reported. We added CAC to the list of pathologies affected by H_4R and ascribed H_4R-mediated histamine function to normal mouse colon epithelial cells. Currently, we aim at gaining mechanistic insight into the function of H_4R on colon epithelial cells and at transferring the results to the human system. Cell lines are versatile models to investigate cellular and molecular mechanisms of a given system. Therefore, we comprehensively screened cell lines of different origin for their histamine receptor mRNA expression profile and their reactivity to histamine stimulation. We provide data indicating, that human colon-derived cell lines mostly express H_1R, while expression of H_2R and H_4R only occurs occasionally. Combining the expression data with functional analyses, we learned that they do not necessarily coincide with one another, questioning the suitability of the tested cell lines for the analysis of histamine / histamine receptor function in IBD.

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Oral Presentations Abstracts

EHRS 2022 Hannover

Oral Presentations

O1

ROLE OF ADENOSINE A3 RECEPTORS (A3AR) IN BLEOMYCIN-INDUCED LUNG FIBROSIS IN MICE

S. Marri, S. Sgambellone, A. Pini, E. Masini, D. Salvemini, L. Lucarini

Lung fibrosis is a progressive disorder, with a poor prognosis, characterized by interstitial fibrosis of the lung as a pathological response to chronic inflammation. Adenosine receptors (AR) are involved in the development of inflammation and fibrotic conditions. Adenosine, through its low affinity A2B receptor, stimulates mast cells to release histamine, and promotes bronchoconstriction and differentiation of lung fibroblasts into myofibroblasts, typical of the fibrotic event. In asthmatic patients the expression of A3AR is mainly in eosinophils and this receptor mediates inhibition of eosinophil activation and chemotaxis, therefore a selective A3AR agonist could be beneficial in the treatment of asthma.

The present investigation has been carried out to explore the action of MRS5980, a highly selective A3AR agonist, in a murine model of lung fibrosis and to study the modulation of mast cell degranulation and histamine release, in order to find a link between A3AR and histamine signaling.

Mice were intratracheally injected with bleomycin and for the successively 21 days they were treated with vehicle or different doses of MRS5980. We investigated the effects of treatments on lung stiffness, studying the airway resistance to inflation; we measured inflammatory markers (TNF-α, IL-1 β, IL-10, IL-6), and TGF-β expression and α-SMA deposition, indexes of fibrosis establishment; moreover, we evaluated mast cell degranulation and histamine release in lung tissue.

Bleomycin administration increased lung stiffness, TGF-β levels, α-SMA deposition and content of oxidative stress and inflammatory markers. On the contrary, MRS5980 attenuated, dose dependently, all the analyzed physiological, biochemical, and histopathological markers and reduces lung mast cell activation.

Our findings support the proposal that A3AR agonists could have a therapeutic potential in reducing the progression of signs and symptoms of the disease by decreasing inflammation, TGF-β expression and fibrotic markers.

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Oral Presentations

O2

INVESTIGATING THE INTERPLAY OF TRP CHANNELS AND HISTAMINE RECEPTORS IN A HUMAN NEURON-KERATINOCYTE CO-CULTURE MODEL

E.J. Wunschel, G. Köther, B. Seeger, W. Bergforth, R. Gutzmer, and T. Werfel

Pruritus, a characteristic symptom of chronic inflammatory skin diseases like Atopic Dermatitis and Psoriasis, is characterized by a complex interplay of neuronal and non-neuronal cells. Important mediators are distinct subfamilies of TRP-channels (transient receptor potential channels), the vanilloid- (TRPV) and ankyrin-subfamily (TRPA). Stimulation of TRP channels on keratinocytes leads to the release of pro-inflammatory substances which can activate sensory neurons. TRPV4 was shown already to play an important role in the mediation of histamine-induced itch. The questions, if TRPV1, TRPV3, TRPV4 and TRPA1 on keratinocytes are involved in histamine-induced pruritus as well, and if there are differences between histamine receptors, are addressed in this study. Therefore, human keratinocytes and iPSC-derived neurons were seeded into the XonaChip®, a microfluidic device which enables fluidic isolation between the different compartments but allows neurite outgrowth through micro channels. Histamine receptor agonists were applied to the keratinocytes and incubated for 6, 24 and 48hrs, respectively. Neurite outgrowth was monitored by immunofluorescence staining and mRNA expression of TRP channels were analyzed by qRT-PCR. Furthermore, the mRNA expression of TRP channels were compared between outer root sheath (ORS) keratinocytes obtained from atopic, psoriatic and healthy donors before and after stimulation with histamine receptor agonists. The increase of intracellular calcium concentrations after stimulation of keratinocytes with histamine receptor agonists was measured with and without pretreatment of TRP channel inhibitors to determine which TRP channels and histamine receptors might be involved in the histamine-induced Ca^{2+}-influx. Initial results will be presented to elucidate the interplay of TRP channels and histamine receptors in human neuronal and non-neuronal cells regarding the emergence of histamine-induced itch.

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Oral Presentations

O3

HISTAMINE INCREASES THE EXPRESSION OF IL-18 AND THE IL-18R ON HUMAN EOSINOPHILS FROM AD PATIENTS

L. Beyer, A. Kabatas, H. Stark, S. Mommert, T. Werfel, R. Gutzmer, K. Schaper-Gerhardt

The histamine 4 receptor (H_4R) has emerged as a relevant target for mediating itch and inflammation in atopic dermatitis (AD). Eosinophils are part of the dermal infiltrate in AD patients and constitutionally exhibit a high basal H_4R expression compared to eosinophils from healthy subjects and other relevant immune cells. The aim of this project was to investigate immunomodulatory functions of H_4R on eosinophils. By means of RNA sequencing, the transcriptome of eosinophils isolated from venous blood of 4 AD patients was analysed after histamine and H_4R stimulation. Subsequently, the expression of two of the top regulated genes, IL-18 and IL-18R, was examined in another 35 AD patients and 22 healthy controls by real-time PCR and flow cytometry. We showed that eosinophils from AD patients upregulate IL-18 and IL-18R mRNA expression by histamine, which confirmed the data from the trancriptome analyses. The IL-18R upregulation was, depending on the cytokine environment, mediated via the H_2R (Th1 environment) and H_4R (Th2 environment), respectively. The increase of IL-18R expression was more pronounced in AD patients compared to healthy controls and the upregulation correlated with the percentage of eosinophils in the blood. The functionality of IL-18R on eosinophils was verified via increased ECP mRNA expression after pre-incubation with histamine and IFN-γ followed by stimulation with IL-18. IL-18 is a pleiotropic cytokine of the IL-1 family and promotes both Th1 and Th2 immune responses depending on the surrounding cytokine milieu. IL-18 serum levels are increased in AD patients and positively correlate with disease severity. Our results indicate an immunomodulatory role of histamine via the H_2R and H_4R in the upregulation of IL-18 and the IL-18R on eosinophils from AD patients. This suggests that H_2R and H_4R are potential therapeutic targets in AD and other inflammatory diseases involving eosinophils.

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Oral Presentations

O4

ANTI-INFLAMMATORY EFFECTS OF LINS01007, A HISTAMINE H_4R ANTAGONIST, IN MICE DSS-INDUCED COLITIS MODEL

B.K. Lippi, G.A.B. Fernandes, G.A. Azevedo, N.G.S. Negreiros, M.A.V. Landgraf, J.P.S. Fernandes, R.G. Landgraf

Ulcerative colitis is a chronic inflammatory bowel disease with growing incidence worldwide. The current drug treatment of ulcerative colitis involves non-steroidal anti-inflammatories, corticosteroids, immunosuppressant and biological drugs, which present considerable adverse reactions and expensive costs. Thus the search for small molecule anti-inflammatory compounds with novel mechanism of action is needed. With this regard, histamine H_4 receptor (H_4R) is a noteworthy target for drug discovery against UC. Since our group reported the compound 5-choro-1-[(2,3-dihydro-1-benzofuran-2-yl)methyl]piperazine (LINS01007) as H_4R antagonist (pK_i 6.2), its effects and pharmacological profile on a DSS-induced mice model of ulcerative colitis were assessed. Experimental acute colitis was induced in male BALB/c mice (n=5-7) by administering 3% DSS in the drinking water for six days. The compound test LINS01007 was administered i.p. (5 mg/kg) daily, and compared with control group without treatment. Body weight, the consumption of water and feed, and the presence of faecal blood were monitored during 7 treatment days. Animals submitted to acute colitis protocol showed significant reduction on water and food intake from the 4th day (p<0.05), with significant weight reduction in these animals. These events were prevented by LINS01007, although the presence of blood in the stool was not reverted. Histological signs of oedema, hyperplasia and disorganized intestinal crypts, as well as neutrophilic infiltrations were found in control mice while these findings were significantly reduced in animals treated with LINS01007. Significant reduction of PGE_2 (but not LTB_4) levels along with COX_2 expression were found in treated animals. Important reduction on IL-6 levels was also observed after treatment with LINS01007. The results showed the noteworthy effects of LINS01007 against DSS-induced colitis, denoting the potential of antagonism of H_4R as promising treatment for this condition.

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Oral Presentations

O5

ROLE OF HISTAMINE H4 RECEPTOR IN CHRONIC DERMATITIS - RESULTS WITH THE H4 RECEPTOR KNOCKOUT MOUSE

Jonathan Vidak, Viviane Filor, Tichakorn Singto, Jenny Wilzopolski, Wolfgang Bäumer

The role of histamine in allergic skin diseases has been re-evaluated with the discovery of the histamine H4 receptor. Histamine is a well-known mediator released in large amounts from e.g. mast cells upon IgE bridging by relevant allergens. It contributes particularly to a Th2 driven inflammatory reaction and activates sensory neurons mainly via histamine H1 and H4 receptors. However, H1 receptor antagonists are only of minor therapeutic value in allergic skin diseases like atopic dermatitis. With the help of H4 receptor knockout mice, we wanted to elucidate the role of the H4 receptor in a chronic allergic dermatitis model driven by the chemical respiratory allergen toluene-diisocynate (TDI), known to induce a Th2 driven allergic skin reaction associated with severe pruritus. TDI was repetitively administered to the mouse ear and back skin and ear thickness as endpoint of inflammation and scratching bouts were measured. After the first two challenges with TDI (days 29 and 32), ear swelling was not different between H4R KO mice and wild type mice, however after the following challenges (days 36, 39 and 41) there was a significant decrease in inflammatory response in H4R KO mice compared to wild type. Pruritus was increased after the first three challenges and then decreased slightly in the following challenges in wild type mice. This pattern also occurred in H4R KO mice and we could not see any significant difference in itch behaviour between wild type and H4R KO mice. These results further indicate a role of H4 receptor on inflammation particularly in the chronic setting of allergic skin diseases.

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Oral Presentations

O6

A NOVEL ANTAGONIST OF HISTAMINE H_4 RECEPTOR, JSJ, AS A POTENTIAL CANDIDATE FOR THE TREATMENT OF NEUROPATHIC PAIN – *IN VIVO* AND *IN VITRO* STUDIES.

Katarzyna Popiolek-Barczyk, Aleksandra Pędracka, Małgorzata Więcek, Dorota Łażewska, Katarzyna Kieć-Kononowicz, Katarzyna Starowicz

Neuropathic pain is a pathology caused by damage or diseases of the somatosensory nervous system. This type of pain is often refractory to standard therapies and become a growing public health problem contributing to a low quality of patients' lives and high costs of medical care. Histamine H_4 receptor (H_4R) has emerged as promising target for pharmacological intervention. The expression of H_4R is strongly related to cells involved in immune responses, such as microglia, which is closely associated with neuropathy development. The aim of our study was to investigate the impact of newly synthesized H_4R antagonist, JSJ, on mechanical (von Frey) and thermal (cold plate, tail flick) stimuli in models of acute and neuropathic (CCI, chronic constriction injury) pain in mice. Moreover, we analysed the impact of JSJ on microglia activation in *in vitro* model (BV-2 cell line). Single injection of JSJ (1, 10, 20 mg/kg, i.p.) dose- and time-dependently reduced symptoms of pain at day 14^{th} after CCI. In chronic treatment paradigm (following day 14^{th}, for 7 days) JSJ (20 mg/kg) also reduced symptoms of neuropathy. Moreover, in healthy animals, JSJ (20 mg/kg) diminished spinal nociceptive responses induced by thermal stimuli. Our *in vitro* studies revealed that JSJ significantly diminished level of pro- (IL-1β, IL-6), but not anti-inflammatory (IL-10), factors in lipopolysaccharide-activated microglia. Our work provides the first evidence for the analgesic potency of novel H_4R antagonist and its influence on microglial cells activation. We hypothesize that the analgesic effects of H_4R antagonists under neuropathic pain might be related to reduced microglia activation and neuroinflammation. We recognize the need for a successful neuropathic pain management; therefore, we believe that our studies may be a breakthrough in the search for innovative pain therapies. Acknowledgements: This work was financed by grant from the National Science Centre, Poland, SONATA 2019/35/D/NZ7/01042.

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Oral Presentations

O7

ROLE OF MAST CELLS IN GASTROENTEROLOGY AND ENDOSCOPY

M. Raithel, K. Hotfiel, H.W. Schultis, A. Gozner, A. Hoerning, R. Rieker

Mast cells are primitive immune cells that appear early in the stomach in animals when the life made the step from water to land. So, they are very early connected with the GI-tract and have since evolved into multifunctional cells in vertebrates. However, clear diagnostic criteria of mast cell hyperplasia or -secretion are lacking for the GI-tract.

Apart from primary mastocytosis (e.g. cutaneous, indolent or systemic form, D816V), the first cases of continuous mast cell activation have been described since 2007. The symptomatology of such Kit-positive and KIT mutation-negative patients was characterized by exploring mucosal mast cell numbers defining thresholds, intestinally produced IgE, ECP, Tryptase and tissue eosinophilia.

Mast cells in the GI-tract are strictly regulated to control physiological functions. There is evidence for the microbiota, neurotransmission and other local or exogenous factors that can intervene with tissue- /mast cell homeostasis, altering their secretion at any time. Ex vivo experiments show abnormal secretion within seconds in the event of deviations from the physiological state (nitric oxide), within minutes (histamine, PAF, TNF) and within hours (cytokines). Segmental or diffuse mast cell activation is very often coupled with intestinal barrier damage, local functional symptoms, a large number of non-erosive IBS symptoms, and sometimes also with peripheral and central changes in nerve function, alterations of blood-brain barrier and the psyche.

The activation of the mast cells can take place via various immunological (IgE, C3, C5 complement), non-immunological (salicylates, contrast media, etc.) and microbial stimuli. By analyzing local antigen-specific IgE concentrations in the gut lumen during endoscopy seronegative GI food allergy could be confirmed, by measuring high tryptase levels in gut lavage intestinal mastocytosis could be established and by special immunohistochemistry of diamine oxidase individuals with disturbed histamine degradation can be separated within the heterogenous population of IBS patients. Local IgE production in gut shows only a weak correlation with serum or skin tests, confirming different immunological compartments.

In addition to emergency medication for acute shock reactions (e.g. adrenaline injector, steroids, H1 and H2 antihistamines), mast cell stabilizers (e.g. disodium cromoglycate), older and newer H1 antihistamines (e.g. ketotifen or desloratadine) and seldom anti-IgE antibodies are used for symptom relief in IBS, GI-food allergy or mast cell activation syndrome.

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Oral Presentations

O8

STABILIZING HISTAMINE RELEASE IN MAST CELLS SIGNALING FROM THE GUT MITIGATES NEUROINFLAMMATION IN THE BRAIN POST STROKE

Maria P. Blasco Conesa, Frank W. Blixt, Pedram Honarpisheh, Bhanu P. Ganesh

Clinically, ~65% of stroke patients are left with functional impairments after stroke and 15% die shortly after their stroke. Increasing evidence suggests that peripheral inflammatory responses after stroke play an important role in determining neurological outcome. Mast cells (MCs) are one of the most rapid responders to injury. MCs release histamine (HA), a pro-inflammatory transmitter that enhances inflammation. Gut MCs are a major source of HA. We hypothesize that stroke in aged animals will lead to robust gut mucosal MC-activation and HA release, with subsequent gut disruption and inflammation. Stabilizing peripheral MCs will decrease peripheral/central inflammation, MC trafficking, and improve stroke outcomes. We used a reversible middle cerebral artery occlusion (MCAO) model of ischemic stroke in aged (18mo) wild-type male mice to investigate the MC role in neuroinflammation post-stroke (PS). We stroke the aged animals and treated the animals with 25 mg/kg BW of cromolyn (MC stabilizer), oral gavage. Cromolyn was administered at 3-h, 10-h, 24-h and every other day PS. Positive control group that were stroked but treated only with saline. In total, four groups, stroke and sham (surgery control), out of these animals one set received cromolyn and one set received saline. We sacrificed animals at 3-h, 24 –h and 3-days after cromolyn treatment post-stroke. We found that cromolyn administration significantly reduced MC numbers in the brain at 24-hours ($P<0.0051$) and 3 days ($P<0.0005$) PS. In association with that we found behavioral changes with improved motor activity at 3-days post-stroke animals after cromolyn treatment.We also found that gutmast cells are significantly reduced after cromolyn treatment in the 24 hours and 3-days PS groups ($P<0.01$). Additionally, we found significant decrease in neurological deficit score at the 3-days PS animals which was not very prominent at 24-hours ($P<0.0125$). GFP+MC introduction via adoptive transfer to c-kit-/- MC knock-out animals showed elevated MC recruitment to the injury site PS. In addition to the reduction in peripheral inflammation, we found rescue effect on the microbiome composition after cromolyn administration that prevented stroke induced dysbiosis. Our results show that preventing MC-HA release post-stroke possess clinical value in preventing neuroinflammation PS.

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O9

TH2 CYTOKINES AND HISTAMINE REGULATE THE EXPRESSION OF ENZYMES IN THE BIOSYNTHESIS OF LEUKOTRIENES, RELEASE OF CYSTEINYL LEUKOTRIENES AND THEIR RECEPTOR EXPRESSION IN HUMAN MAST CELLS

Patricia Gehlhaar, Katrin Schaper-Gerhardt, Ralf Gutzmer, Thomas Werfel, Susanne Mommert

In skin lesions of atopic dermatitis (AD), a chronic inflammatory skin disease, mast cells beyond other immune cells are present in increased numbers. Upon activation, mast cells release a plethora of mediators, in particular histamine and leukotrienes, as well as chemokines and cytokines, which modulate the immune response of cells in their microenvironment which may influence mast cells in an autocrine loop.

This study aimed to investigate the effects of Th2 cytokines and histamine on the generation of enzymes in the biosynthesis of leukotrienes, on the release of cysteinyl leukotrienes (CysLTs) as well as on the expression of their receptors on human mast cells from healthy control persons and from patients with AD.

Human mast cells were generated from CD34 positive progenitor cells and cultured in the presence of IL-3 for five weeks. Mast cells expressing the high affinity receptor for IgE (FcεRI) and CD117 (c-kit) were stimulated with IL-4, IL-13, histamine and different histamine receptor selective ligands. The expression of enzymes in the biosynthesis of leukotrienes and expression of CysLT receptors were quantified by qPCR. The release of CysLTs was measured by ELISA.

5-Lipoxygenase (5-LO) and the 5-LO-activating protein showed increased mRNA expression in untreated, IL-4, IL-13 or histamine treated human mast cells from AD patients when compared to healthy controls. Histamine and Th2 cytokines up-regulated leukotriene C4 synthase mRNA expression, induced the release of CysLTs and up-regulated mRNA expression of CysLT receptors in human mast cells from healthy controls and AD patients.

In conclusion, we provide evidence that in an acute allergic situation Th2 cytokines and histamine activate the expression of enzymes in the biosynthesis of leukotrienes resulting in an enhanced release of CysLTs and that histamine up-regulates CysLT receptor expression. This suggests a novel mechanism for sustaining mast cell activation by an autocrine loop.

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Oral Presentations

O10

EXPRESSION AND REGULATION OF THE CYSTEINYL LEUKOTRIENE SYSTEM IN HUMAN MONOCYTES AND MACROPHAGES BY CYTOKINES PLAYING A ROLE IN ATOPIC DERMATITIS

S. Lietzau, S. Mommert, T. Werfel

Elevated levels of interleukin-4 (IL-4), interleukin-13 (IL-13) (acute phase) and IFN-γ (chronic phase) are present in skin lesions of atopic dermatitis (AD). Cysteinyl leukotrienes (CysLTs) mediate itch and skin fibrosis. CysLT synthesis involves the 5-lipoxygenase (5-LO), the 5-lipoxgenase activating protein (FLAP) and the leukotriene-C4-synthase (LTC4S).

Our aim was to analyze the effects of the Th2 cytokines IL-4 and IL-13 as well as of IFN-γ by comparing 5-LO, FLAP and LTC4S mRNA expression and the release of the CysLTC4 in monocytes and macrophages from healthy controls and AD patients.

Monocytes were isolated from PBMCs from healthy donors and AD patients. Macrophages were generated from monocytes by cultivation in macrophage colony stimulating factor for 8 days. Monocytes and macrophages were stimulated with IL-4, IL-13 and IFN-γ. 5-LO, FLAP and LTC4S mRNA expressions were quantified by qPCR. CysLTC4 release was measured by ELISA.

Constitutive mRNA expressions of 5-LO, FLAP and LTC4S were highly elevated in monocytes from AD patients. 5-LO mRNA expression was upregulated by IFN-γ and downregulated by IL-4 and IL-13 in monocytes and macrophages. LTC4S mRNA expression was downregulated by IFN-γ but upregulated by IL-13. FLAP mRNA expression was downregulated in monocytes and macrophages from both cohorts by all cytokines. Constitutive release of CysLTC4 was elevated in macrophages from AD patients. CysLTC4 release was increased in macrophages from healthy donors after IL-4 stimulation.

Our findings indicate that IFN-γ specifically promotes the 5-LO catalyzed step of CysLT synthesis, whereas IL-4 and IL-13 only stimulate the LTC4S dependent part. Further research is needed to characterize other products catalyzed by 5-LO under IFN-γ influence and to identify triggers for CysLTC4 release from IL-13 stimulated monocytes and macrophages.

In conclusion, elevated levels of 5-LO, FLAP and LTC4S mRNA expression in monocytes from AD patients and an elevated CysLTC4 release from macrophages from AD patients suggest a relevant role of CysLTs derived from these cells in AD pathogenesis.

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Oral Presentations

O11

HISTAMINE H_4 RECEPTOR IS EXPRESSED IN HUMAN T-CELL LYMPHOMA AND ITS ACTIVATION INDUCES ANTITUMOR EFFECTS

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The discovery of the human histamine H_4 receptor (H_4R) has contributed to our understanding of the role of histamine in numerous physiological and pathological conditions, including tumor development and progression. The lymph nodes of patients with malignant lymphomas have shown to contain high levels of histamine, however, less is known regarding the expression and function of the H_4R in T-cell lymphoma (TCL). The aims of the present work were to investigate the expression of the H_4R in TCL, and to evaluate the potential antitumor effects of histamine and H_4R ligands. Using RNAseq available data from different studies, we show the mRNA expression of H_4R in human TCL samples. We validated the expression of H_4R isoforms (mRNA and protein) in human aggressive TCL cell lines (OCI-Ly12, Karpas 299, and HuT78). The full length H_4R and the two alternatively spliced isoforms were differentially expressed in the three TCL cell lines. Histamine and specific H_4R agonists (VUF8430 and JNJ28610244) significantly reduced cell viability in a dose-dependent manner ($P<0.05$). The combined treatment with the H_4R antagonist (JNJ7777120, 10 µM) reversed the effects of the H_4R ligands. Importantly, we screened a drug repurposing library of 433 FDA-approved compounds (1 µM) in combination with histamine (10 µM) in Hut78 cells. Histamine produced a favorable antitumor effect with 18 of these compounds, including the histone deacetylase inhibitor (HDACi) panobinostat. Apoptosis, proliferation, and oxidative stress studies confirmed the antitumoral effects of the combination. In conclusion, the H_4R is expressed in TCL and histamine could be an attractive compound for its use as a single agent or in combination with HDACi for the treatment of TCL.

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O12

HISTAMINE AND HISTAMINE H_3 RECEPTORS ARE INVOLVED IN THE SYNTHESIS OF IL-17 IN PSORIASIS.

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Psoriasis is a chronic, immune-mediated, inflammatory disease that is pathogenically driven by proinflammatory cytokines. Immunologic role of interleukin (IL)-17, the major effector cytokine in the pathogenesis of psoriatic disease. IL-17 family consists of five other members (IL-17B-F) and recently it have been demonstrated that this cytokine and its receptors are a targets in the effective treatment of psoriasis and psoriatic arthritis. Within the skin and joints, IL-17A acts on cellular targets, including keratinocytes, neutrophils, endothelial cells, fibroblasts, osteoclasts, chondrocytes, and osteoblasts, to stimulate production of various antimicrobial peptides, chemokines, and proinflammatory and proliferative cytokines. Data on the role of histamine in psoriasis, remain controversial. Some studies share the opinion that histamine is not involved in pruritus associated with psoriasis and histamine plasma level are low. On other hand it was observed that skin mast cells are hyperactivated in active psoriasis. Thus, it is possible that histamine can be overproduced locally in the dermis and the histamine plasma level does not necessarily reflect its content in the skin. While most attention in the most of previous studies was focused on the H_1R, other histamine receptor subtypes should not be overlooked. The previous investigations suggest that psoriasis and co-morbidities, especially obesity may have a common chronic inflammatory mechanisms. The goal of this study assesses the role of histamine and its H3 receptors in the synthesis of IL-17A in psoriasis in patients with different body mass index (BMI). 59 patients with psoriasis with varied skin lesions and severity and 38 healthy donors were assessed. The concentrations of IL-17A in supernatants of 48h-cultivated PBMC from psoriasis patients and healthy volunteers were examined by ELISA. To assess the impact of histamine, PBMC were cultivated in the presence of histamine and the histamine and H_3/H_4antagonist (Ciproxifan).The study showed more than 2 fold increase (~60%) in the production of IL-17A in patients with psoriasis in comparison to healthy donors ($p<0.05$). Moreover the synthesis of IL-17 was higher in cell culture of PBMC of patients with increased BMI. Further experiments demonstrated that histamine highly increases (up to 60%) the production of IL-17 by cultivated PBMC both of healthy donors and psoriasis patients. This effect of histamine was diminished in cell cultures pre-cultivated in the presence of H_3/H_4 antagonist and it was were more pronounced in patients with high BMI than in patients with normal BMI (21-25). Thus, the study demonstrated that histamine and histamine H_3 receptors are involved in the regulation of IL-17A synthesis in psoriasis patients and the intensity of this effect highly depend on the co-morbid metabolic disorders. *Peoples´Friendship University of Russia (RUND University, Miklukho-Maklaya Str. 6, Moscow, 117198, Russian Federation* khanfer1949@gmail.com

Oral Presentations

O13

HISTAMINE H3 RECEPTOR INVERSE AGONISTS/ANTAGONISTS: NEW THERAPEUTIC AGENTS FOR THE TREATMENT OF EXCESSIVE DAYTIME SLEEPINESS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA

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Histamine was first identified in the central nervous system about 50 years ago, but its role in the regulation of sleep/wake behaviour through H3 receptors, has been uncovered only in the last 3 decades. Histamine H3 receptor (HR3) antagonists enhance wake at the expense of both NREM and REM sleeps and can reduce the abnormal direct transition from wake to REM sleep in orexin$^{-/-}$ mice, a model of human type I narcolepsy. Therapeutic application for this phenomenon was reached with the approval by the EMA (2016) and the FDA (2019) of pitolisant as a first in class H3R antagonist for the treatment of excessive daytime sleepiness (EDS) and cataplexy attacks in narcoleptic patients.

EDS symptoms are also present in patients suffering from obstructive sleep apnoea (OSA). The first line therapy consists with the use of devices that maintain a continuous positive airway pressure (CPAP), thus normalizing the apnoea-hypopnoea index, suppressing the nocturnal O_2 desaturation and reducing the sleep fragmentation. Overall CPAP reduces EDS and improve vigilance, cognition, and quality of life. However, about ~15% of patients do not tolerate CPAP, and 20-30% discontinue its long-term use. Significantly, 15% using CPAP still present residual EDS. Hence, the efficacy and safety of pitolisant were assessed in the treatment of EDS in these patients.

After phase II studies showing positive signals in reducing EDS in OSA patients, two phase III studies with a randomized double-blind design, placebo-controlled, parallel-group, multi-centre trials assessed the effects of pitolisant (titration up to 20 mg) in the treatment of EDS in OSA patients treated by CPAP or refusing it, over a 12-week period. The 1^{ary} endpoint was the mean change in the Epworth sleepiness score (ESS), and the main 2^{ary} endpoints were mean sleep latency (OSleR test), Pichot fatigue score, sleep diary variables, clinical global impression, and patient's global opinion.

The metanalysis of results evidenced that pitolisant leads to a significant reduction in the ESS by 3.1 (95%CI [4.1, -2.1], p<0.001) as well as changes in additional secondary endpoints (*e.g.*, improvement of the OSleR test value +1.18 (95%CI [1.02, 1.35], p=0.022). These data lay the ground for the approval by the EMA of pitolisant (Ozawade®) for the treatment of EDS in OSA patients in September 2021.

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Oral Presentations

O14

SELECTIVE AND PERIPHERALLY ACTING HISTAMINE H_3 RECEPTOR (H_3R) ANTAGONIST PF-0868087 PRODUCES ANALGESIC EFFECT IN NEUROPATHIC PAIN VIA THE MAMMALIAN TARGET OF RAPAMYCIN COMPLEX 1 (mTORC1) PATHWAY IN MICE

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H_3R has been targeted for several diseases including neuropathic pain that results from damage to or disease within the somatosensory system. Despite evidence supporting H_3Rs expression in nociceptive pathways, many of the findings reporting the functional implication of H_3Rs in chronic pain have been contradictory. Recent development of novel compounds targeting H_3R have provided interesting tools to revisit their role in chronic pain, particularly at the level of peripheral nervous system. Indeed, we recently demonstrated the analgesic efficacy of a novel, selective and peripherally acting/centrally-sparing H_3R antagonist, PF-0868087, after systemic (i.p.) administration in nerve injury-induced neuropathic pain in mice. Here, we extended our analysis of the effects produced by PF-0868087 and we examined potential mechanism underlying PF-0868087-induced analgesia. Using the immunoblotting technique, we assessed the activity of the mTORC1 pathway that regulates protein synthesis via controlling the activation of specific downstream effectors such as S6 ribosomal proteins (S6RP) and eukaryotic initiation factor 4E-binding protein (4E-BP1). We observed that i.p. administration of PF-0868087 (10 mg/kg) once daily for 4 consecutive days (from day 7 to day 10 post injury) significantly reduced the ratio of phosphorylated to total S6RP ($t_{(9)}$=3.4, P<0.05, n=5-6) and 4E-BP1 ($t_{(9)}$=2.7, P<0.05, n = 5-6) on the ipsilateral side of the spinal cord in neuropathic mice compared to vehicle treated animals. In addition, a significant inhibition of phosphorylation of S6RP ($t_{(10)}$=5.7, P<0.05) was detected on the ipsilateral side of sciatic nerve in neuropathic mice treated with PF-0868087 as described above. These effects were not observed on the contralateral side or in sham controls. Taken together, our results provide first evidence of a potential involvement of H_3Rs in the regulation of chronic pain through histamine-independent mechanisms directly involving the mTORC1 pathway.

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Oral Presentations

O15

A BRIEF HISTORY OF HISTAMINE NEUROPHYSIOLOGY

Helmut L. Haas, Olga A. Sergeeva

Since the introduction of “antihistamines” almost a century ago a waking action of brain histamine has been suggested, 50 years later neurophysiological investigations using highly localized applications of neuroactive substances in the immediate environment of recorded neurones by microelectrophoresis of suspected transmitters and interacting drugs from multibarrel electrodes was the most popular method for functional identification of neurotransmitters.

Microelectrode-recording and substance application in experiments from the 1970s are shown from brainstem, cortex and hypothalamus: Inhibition of neuronal firing in the brainstem, the effect of metiamide on responses to GABA and histamine. The functional identification of recorded neurons is a challenge: response to saline for identification of a supraoptic neuron responding to histamine. Histaminergic transmission occurs also in the fly eye and Aplysia, whose large histaminergic neuron projects to follower cells and elicits postsynaptic potentials. The recording from brain slices in perfusion chambers allows intracellular and patch-clamp recordings in vertebrate, even human neurons. H1R mediate excitation through inward currents, responsible for cortical arousal.

The hippocampus provides mechanisms of synaptic plasticity, learning and memory: H2R activation induces long-term potentiation through cyclic AMP. Histamine also potentiates the NMDA current in response to glutamate. Recordings from slices containing the tuberomamillary nucleus demonstrate H3-autoreceptor actions.

In recent years, we record red identified histamine neurons from a HDC-Tmt reporter mouse and new technologies allow analysis of the single cell transcriptome obtaining mRNA through the recording electrode. Combination of electrophysiology with optical recording, tracing and whole transcriptome analysis of individual neurons by next generation sequencing (NGS) will further characterize the whole system and its functional domains.

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Oral Presentations

O16

REDUCED NUMBERS OF CORTICOTROPIN-RELEASING HORMONE NEURONS IN NARCOLEPSY TYPE 1

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Narcolepsy type 1 (with cataplexy) is a rare invalidating chronic sleep disorder caused by a loss of hypocretin neuropeptides, presumed to be due to an auto-immune process. A systematic search for possible involvement of other hypothalamic neurons implicated in sleep-wake regulation has never been performed.

We systematically quantified immunohistochemically stained sleep-wake related neuronal populations and the presence of microglia in the hypothalamus of narcolepsy type 1 (n=4) and matched controls.

Biological clock: there was no difference in the numbers of vasopressin-expressing neurons in the suprachiasmatic nucleus. Sleep promoting neurons: the density of galanin positive neurons in the ventrolateral preoptic nucleus was stable. Arousal related neurons: we confirmed the hallmark loss of hypocretin-1 expressing neurons and the increased numbers of histaminergic neurons. The density of choline acetyltransferase-expressing neurons in the nucleus basalis of Meynert was unchanged. We found a selective and strong reduction in the number of corticotropin-releasing hormone (CRH)-positive neurons in the paraventricular nucleus (PVN) of narcolepsy type 1 and significant less CRH-positive fibers in the median eminence. While, no alteration was observed in other PVN neurons, i.e. vasopressin, oxytocin, or tyrosine hydroxylase--expressing neurons. Microglial reactions: The presence of ionized calcium binding adaptor molecule 1 tended to be increased in the hypocretin area, but not in any other adjacent area. The human leukocyte antigen-staining was similar in all these areas.

This surprising decrease in CRH neurons may contribute to sleep-wake symptoms and may provide novel targets for diagnostics and interventions.

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Oral Presentations

O17

ST-2223, A NOVEL MULTIPLE ACTIVE H3R/D2R/D3R ANTAGONIST MODULATES BRAIN NEUROTRASMITTERS AND ENHANCE SOCIAL INTERACTION IN BTBR MOUSE MODEL OF AUTISM

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Neurotransmitters displayed significant roles in initial brain development, hence neurotransmitter dysregulations may be highly implicated in the pathophysiology of brain disorders including Autism Spectrum Disorder (ASD). Given the fact that there are no FDA approved effective treatments for the social deficits in ASD, the effects of chronic treatment with the novel multiple-active H3R/D2R/D3R antagonist ST-2223 (2.5, 5, and 10 mg/kg, i.p.) on social behaviors and anxiety level in male Black and Tan BRachyury (BTBR) mice were evaluated using social approach test and open field locomotor test respectively.

ST-2223 (2.5, 5 and 10 mg/kg, i.p.) dose-dependently attenuated sociability and social novelty deficits of BTBR mice (P<0.05). Moreover, ST-2223 restored disturbed anxiety level observed in BTBR, whereas the reference drug aripiprazole (1 mg/kg, i.p.) failed to correct the anxiety behaviors but rescued the hyperactivity measures of BTBR mice. Furthermore, tissue levels of monoaminergic neurotransmitters quantified by LC-MS/MS in four brain regions including prefrontal cortex, cerebellum, striatum, and hippocampus revealed that following the administration of ST-2223 (5mg/kg), significant elevation of histamine in cerebellum and striatum; dopamine in prefrontal and striatum; as well as acetylcholine in prefrontal cortex, striatum and hippocampus(all P < 0.05) in BTBR mice were observed.

These results provide evidence that modulation of brain neurotransmission represented by chronic administration of multiple-active H3R/D2R/D3R antagonist ST-2223 may serve as an effective pharmacological therapeutic target to rescue social deficits in BTBR animal model of ASD. Further studies on neurochemical alterations in ASD are crucial to elucidate the underlying mechanisms of social deficits and providing evidenced-based intervention strategies that may improve the social acceptability of ASD, and other social interaction associated disorders.

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Oral Presentations

O18

OLHA MODULATES ACTIVITY OF MOUSE BRAIN HISTAMINERGIC NEURONS

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Neurodegenerative diseases are characterized by the pathological increase in brain oleic acid (OLA), which can conjugate to biogenic amines. We have previously shown that a conjugate between OLA and dopamine (OLDA) modulates firing of brain histaminergic (HA) neurons controlling wakefulness. Here we test the action of a highly purified condensate between OLA and histamine (N^{alpha}-oleoylhistamine, OLHA) on electrophysiological activity and intracellular calcium^{2+} levels of mouse HA neurons in brain slices. Interaction with receptors is studied with the [^{3}H]-N^{α}-methylhistamine displacement assay on HEK293 cells stably expressing the human H3 receptor and with the *in silico* modelling. OLHA bidirectionally modulates the firing of HA neurons. At 10nM OLHA inhibits or has no action, whereas at 1µM it evokes excitatory and inhibitory responses. Inhibition is not seen in presence of the histamine receptor H3 (H_3R) antagonist clobenpropit and in calcium-free medium. Pre-incubation with a histamine-reuptake blocker prevents the decrease in firing by OLHA. OLHA-evoked increase in firing (EC_{50} ~44nM) is insensitive to blockers of cannabinoid 1 and 2 receptors and of the capsaicin receptor, but is significantly impaired by the peroxisome proliferator-activated receptor-alpha (PPAR-alpha) antagonist MK886, which suppresses also the rise in intracellular calcium level caused by OLHA. The OLHA-evoked excitation is mimicked by synthetic PPAR-alpha agonists (gemfibrozil and GW7647) and is abolished by the PKA inhibitor H-89. The H_3R affinity (Ki) for histamine, measured in HEK293 cells is higher than for OLHA (Ki: 42 vs 310nM, respectively). Molecular modelling of PPAR-alpha bound to either OLHA or OEA reveals similar binding energies. These findings shed light on a novel biotransformation product of histamine which may play a role in health and disease as a potent PPAR-alpha activator and modulator of histaminergic transmission.

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Oral Presentations

O19

LYSERGIC ACID DIETHYLAMIDE IS A FULL AGONIST AT HUMAN CARDIAC H_2-HISTAMINE RECEPTORS

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Lysergic acid diethylamide (LSD), is currently used experimentally in psychiatry to treat depression. The mechanism of this effect is unknown. LSD has hallucinogenic effects that are probably mediated via 5-HT_{2A}-serotonin receptors. In clinical studies, LSD was noted to increase the heart rate of human subjects, but it remains unclear whether this is a direct cardiac effect of LSD or an indirect effect via the central nervous system and stimulation of the sympathetic nerve system. However, LSD was shown be a partial agonist at H_2-histamine receptors in rabbit or guinea pig cardiac preparations but the cardiac effects of LSD on human H_2-receptors have never been studied. We tested the hypothesis that LSD stimulates human H_2-receptors in the heart. We studied the effects of LSD in transgenic mice overexpressing the human H_2-receptor (H_2-TG) and their non- transgenic littermates (WT). We measured a positive inotropic and a positive chronotropic effect of LSD starting at 1 µM, reaching a maximum at 10 µM in left atrial preparations or right atrial preparations in H_2-TG, respectively (n=5, $p<0.05$) but not WT. These contractile effects of LSD in atrial preparations from H_2-TG were accompanied by an increase in the rate of relaxation and shortening of the time of relaxation and could be reversed by application of 10 µM cimetidine. The maximum inotropic of LSD could not be further stimulated by additionally applied histamine (10 µM). Likewise, LSD increased force of contraction and phosphorylation at serine 16 of phospholamban in freeze-clamped left atrial preparations from H_2-TG but not WT, which was attenuated by 10 µM cimetidine (n=5). In conclusion, LSD could increase force of contraction by stimulating as a full agonist the human cardiac H_2-receptors and subsequent phosphorylation of phospholamban. The tachycardiac effects of LSD might be due to direct stimulation of H_2-receptors in the sinus node of humans.

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O20

ASSESSMENT OF MULTITARGETING COMPOUNDS FOR HISTAMINE/ DOPAMINE RECEPTOR SUBTYPES AND CHOLINESTERASES

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The role of histamine in cognitive processes, especially involving the H_3 receptor (H_3R) enables the exploitation of its pharmacology for designing drugs against cognitive impairment. In order to sum forces for this objective, the design of multitargeting compounds to bind at dopamine D_3 receptor (D_3R) and cholinesterases (ChEs) beyond H_3R is especially interesting. Blocking H_3R and D_3R along with ChEs may provide clinical advantages over ChEs inhibition alone by providing a network effect on cognition and not only by increasing acetylcholine levels. With this regards, we designed and synthesized a set of 21 compounds as multitargeting agents for H_3R, D_3R, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The aryl-alkyl-piperazine derivatives were synthesized through conventional organic reactions for obtaining ethers, esters and amide derivatives with moderate to high yields. The assessment of the compounds at H_3R and D_3R was done by radioligand displacement assays at maximum concentration of 1 µM while maximum concentration for the AChE and BChE assays, carried out using Ellman's method, was done at maximum concentration of 100 µM. The most promising compounds were then evaluated in full concentration-response curves to estimate the K_i and IC_{50} values. Results showed that several compounds were ligands at H_3R (10), D_3R (6), AChE (3), and BChE (9). The compound LINS01014 is highlighted due to its affinities at all targets (K_i H_3R 1.1 µM; D_3R 3.1 µM; IC_{50} AChE 97.8 µM; BChE 43.7 µM) and another two compounds showed to bind at three targets (LINS05006 = K_i H_3R 2.8 µM; D_3R 0.7 µM; IC_{50} BChE 26.3 µM and LINS05030 = K_i H_3R 0.27 µM; IC_{50} AChE 57.2 µM; BChE = 97.7 µM). These preliminary results from this short list suggest that the methylpiperazine moiety increased the affinity at both AChE and BChE, as well as the selectivity over the H_4R/D_2R. Furthermore, the 4-pyridylpiperazine moiety mostly increases the affinity at H_3R, but decreases the affinity at D_3R.

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O21

PHARMACOLOGICAL RATIONALE FOR USE OF A COMBINATION OF PIRACETAM AND CINNARIZINE (NOOTROPARIZINE) FOR POST-CONCUSSION SYNDROME: A CASE STUDY

K. Hind, M-J. Howes and P.L. Chazot

A male aged 40 years presented with headaches, nausea, vertigo, disturbed vision, photophobia, and extreme fatigue following a road traffic accident (RTA). The patient was also a former international rugby player with a history of sport-related concussions and experiencing short term memory loss over his 20-year career. He had two prior clinically-diagnosed concussions (including one episode of loss of consciousness), and an estimated total of 20 non-reported concussions during his career. His last concussion was sustained 5 years prior to the RTA.

Piracetam is a nootropic drug, which increases cerebral blood flow, stabilises dilatation of the mean capillary diameter, and reduces the number of non-functioning capillaries in the brain cortex. The drug has also been associated with analgesic and neuroprotective effects, and is well-tolerated, with few side effects. Cinnarizine is a piperazine derivative, first generation anti-H_1 histamine receptor, and L-type voltage-gated calcium channel blocker, which restricts the amount of calcium entering cardiac, smooth muscle and some brain cells. The drug is fast-acting, primarily by causing the blood vessels to vasodilate, improving oxygen supply to the heart and brain. It is used to treat cerebral stroke, cerebral arteriosclerosis, and is commonly prescribed for vertigo nausea and vomiting from motion sickness, with minimal side effects. Given the multiple properties and fast-acting nature of Cinnarizine, it is plausible that combining this drug with Piracetam can enhance and accelerate the treatment of PCS.

At month 7 post presentation, the patient halted analgesics and was initiated on combined Piracetam and Cinnarizine (x 3 doses daily). The chemical composition of the two drugs were confirmed using GC-MS methods. The first dose was taken at lunchtime with one glass of water and rapid and complete headache relief was reported to occur within 60 minutes. This was described by the patient as a warm, pleasant feeling in the head and a release of head pressure. Following 3 months of treatment, the patient had made a complete recovery, and returned to full time employment, with no recurrent symptoms reported in the subsequent 4 years.

In conclusion, in our case study, the patient responded extremely favourably and rapidly to a rational combined medication approach of Piracetam and Cinnarizine, with complete resolution within 3 months. This evidence is underpinned by basic science and pharmacological rationale, and warrants further clinical investigation.

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O22

NANOMICELLAR FORMULATION OF PACLITAXEL AND HISTAMINE AS A POTENTIAL BREAST CANCER CHEMOTHERAPEUTIC SYSTEM

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Breast cancer is the most frequently diagnosed neoplasia and a leading cause of cancer related death in women worldwide. Triple negative breast cancer (TNBC) is an aggressive subtype, associated with poor prognosis. Paclitaxel (PTX) is a first-line therapy for TNBC and has low water solubility, poor permeability, and produces severe adverse effects, which limit its clinical use. The aim of this work was to improve the therapeutic index of PTX through the design of combined strategies. For that purpose, we developed nanomicellar polymeric formulations of Soluplus® (S) surface decorated with glucose residues (SG) co-loaded with histamine (HA, 5 mg/mL) and PTX (4 mg/mL). The micellar size, evaluated by dynamic light scattering, showed a hydrodynamic diameter between 80 and 100 nm for loaded nanoformulations with a unimodal size distribution. Cytotoxicity and apoptosis assays were performed to assess their efficacy *in vitro* in human MDA-MB-231 and murine 4T1 TNBC cells. Results showed that histamine improved the antitumoral activity of free PTX and Genexol® (commercial micellar-based PTX-nanoformulation) at low concentrations (0.01-0.1 uM) in both cell lines ($P<0.05$). HA-PTX co-loaded SG micelles exhibited enhanced reactive oxygen species' production, and cytotoxic and pro-apoptotic effects compared to PTX loaded SG micelles, Genexol®, and free PTX ($P<0.05$). Interestingly, histamine reduced PTX-cytotoxic effects on HBL-100 non-tumorigenic breast cells.

^{99m}Tc-radiolabelled SG micelles' distribution was analyzed using *gamma* camera imaging in the TNBC model developed in BALB/c mice with 4T1 cells, showing tumor uptake. Importantly, the *in vivo* studies showed that histamine, both in combination with free PTX and in the HA-PTX loaded SG micellar system, reduced neovascularization and PTX-associated cardiotoxicity. We conclude that histamine enhances the efficacy of nanotechnology based PTX therapy, representing a promising approach for TNBC treatment.

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Oral Presentations

O23

A NOVEL TRANSGENIC MOUSE TO STUDY CARDIAC EFFECTS OF HISTAMINE H_1 RECEPTORS

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It is controversial, whether H_1-histamine receptors (H_1R) affect force of contraction (FOC) in mammalian hearts. In isolated human atrial preparations (HAP), absent, positive (PIE), or negative inotropic effects (NIE) of H_1R stimulation with histamine (HIS) as the agonist and mepyramine (MEP) as the H_1R antagonist have been reported. To better understand the effects of H_1R as a novel tool, we have generated several founders for specific cardiac overexpression of H_1R (H_1-TG). In left atrial (LA) contraction experiments, we noted that in some H_1-TG, but not wild type litter mates (WT), an initial NIE (by 29 ± 8 %) followed by a slowly developing PIE (by 141 ± 11 %, n=5, $p<0.05$) of HIS (from 1 µM to 100 µM). Both effects were antagonized by MEP (up to 100 µM, n=3). HIS increased the beating rate in spontaneously beating right atrial preparations from H_1-TG (157 % ± 15, n=6), sometimes leading to atrial fibrillation. In the presence of 10 µM MEP, additional HIS (up to 10 µM) failed to affect FOC, suggesting that both PIE and NIE of HIS were H_1R-mediated in LA from H_1-TG. The FOC in LA by H_1-TG was affected in the presence of 10 µM suprahistaprodifen (SUP-HIS). Like with HIS, an initial NIE was followed by an PIE. MEP (100 µM) was able to antagonize this effect. The expression of H_1R in H_1- TG atrium was confirmed biochemically by autoradiography with [^{3}H]-MEP. There were no differences between H_1-TG and WT in histological detection (HE staining). Importantly, SUP-HIS (10 µM) increased FOC in HAP by 163 ± 16 % (n=4, $p<0.05$). This increase was antagonized by MEP (100 µM). Moreover, in the presence of 30 µM cimetidine, HIS (100 µM) still increased FOC by 135 ± 11 % in HAP (n=3) and this PIE in HAP was abrogated by MEP (100 µM). We suggest that H_1R can increase FOC in human atrium and in H_1-TG. The clinical role of H_1R in the heart for inotropy and arrhythmogenesis warrants further study.

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Oral Presentations

O24

MOLECULAR MODIFICATIONS ON BASIC GROUP AND AROMATIC MOIETY REVEALED IMPORTANT INFORMATION OF STRUCTURE-AFFINITY RELATIONSHIP FOR LINS01 COMPOUNDS

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In the last years our group had explored the 1-[(2,3-dihydro-1-benzofuran-2-yl)methyl]piperazines (LINS01 series) as histamine H3 and H4 receptor (H3R/H4R) ligands designed based on the JNJ-7777120 compound, an indolecarboxamide-piperazine with nanomolar H4R ligand. In order to eliminate the chirality and get information regarding the piperazine moiety as a basic group in these compounds, modifications were performed to increase the structure-affinity relationship data. A set of 10 compounds were designed by performing ring modifications, homologation and simplification strategies, and synthesized therefore. These compounds were assessed through binding assays at the H3R and H4R, showing notable preference towards the H3R with binding affinities in micromolar and submicromolar range (p*K*i = 5.0-7.0). Only two homopiperazine compounds (LINS01h017 and LINS01h018) exhibited improved (but still low) affinity to the H4R (p*K*i ~5.2) than their piperazine counterparts. The aromatization of the dihydrofuran moiety to remove chirality led to compounds with comparable but lower affinity in some cases. Interestingly, the homopiperazine analogues (LINS01h) showed slightly improved affinities, as well as the ethylenediamine (LINS01e) counterparts. The replacement of the dihydrofuran to anilide led to compounds with lower affinity (p*K*i ~5.3), denoting the importance of this motif to the affinity of LINS01 compounds. Compound LINS01h007 should be highlighted in this series regarding its high affinity to the H3R (p*K*i = 7.02), although its affinity to the H4R (p*K*i < 5.00) was decreased in comparison to its homologue LINS01007 (p*K*i = 6.07). The results added important information for further development of H3R/H4R ligands.

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Oral Presentations

O25

ANTITUMORAL PROPERTIES OF NOVEL HISTAMINE H_3 RECEPTOR ANTAGONISTS FOR BREAST CANCER TREATMENT

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We have reported the expression of the histamine H_3 receptor (H_3R) in human benign and malignant lesions, and cell lines derived from human mammary glands. Its expression is highly correlated with proliferation in breast cancer specimens.

In this work, we aimed at investigating the potential antitumoral activity of 4 novel H_3R antagonists, 1-(2,3-dihydro-1-benzofuran-2-yl)methylpiperazines (LINS01 compounds), which showed high affinity for the human H_3R and selectivity over other histamine receptors. Cell viability and proliferation were assessed by cell titer blue assay and colony formation in human MDA-MB-231 and murine 4T1 triple negative breast cancer cells. Cell apoptosis was assessed by Annexin V staining and flow cytometry together with TUNEL assay, while cell migration was evaluated by wound-healing assay and transwell system.

Results indicate that compounds LINS01009, LINS01010, LINS01022 and LINS01023 (0.01-100 µM) produced a concentration-dependent inhibition on cell growth. The highest responses were observed for LINS01022 and LINS01023, showing an IC_{50} of 9.9±1.1 and 5.2±1.2 µM for MDA-MB-231 cells, and 11.1±1.3 and 3.4±1.2 µM for 4T1 cells, respectively, in the clonogenic assay. These effects were partially reversed by the selective H_3R agonist (*R*)-alpha-methylhistamine.

LINS01022 and LINS01023 (25-50 µM) induced cell apoptosis (3 to 7-fold-increase) and suppressed cell migration in both cell lines (ANOVA, $P<0.01$).

These more potent allylpiperazine compounds at H_3R also exhibited higher antiproliferative and proapoptotic effects than their corresponding methylpiperazine analogues LINS01009 and LINS1010, respectively.

We conclude that the H_3R is involved in the regulation of breast cancer cell growth and progression, offering novel therapeutic potentials for H_3R antagonists.

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Oral Presentations

O26

PHARMACOLOGICAL CHARACTERIZATION OF H_1R AND H_3R SMALL MOLECULE PHOTOLIGANDS – REACHING BEYOND THE UV SPECTRUM

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Photopharmacological modulation of G protein-coupled receptors (GPCRs) offers new exciting ways to modify ligand on-target activity in a spatio-temportal way. One way to do this is introducing a photosensitive moiety to a pharmacologically active compound, hereby hindering its activity (*photocaging*). The active compound can then be irreversibly *uncaged* with light. On the other hand, having a photosensitive moiety (e.g. azobenzene), that can reversibly be modified with light, allows controlled toggling between an *on-* and *off-state* of a given molecule (*photoswitching*). However, most researched photoligands rely on UV light for photo-modulatory effects, which can be harmful for living cells. In addition, despite being lower in energy, longer wavelengths provide better tissue penetration, hereby opening doors for more efficient real-time photo-modulation of ligand activity. An in-house synthetized, red-shifted H_1R photocaged antagonist, VUF25549 is shown to have more than 50-fold lower affinity than its uncaged counterpart, and can be uncaged in live-cell confocal imaging assays. In addition, we present a series of H_3R photoswitches, with photosensitive moieties ranging from UV to visible red light spectrum, and assess how these moieties influence their H_3R binding affinity. Our research shows not only that small histamine photoligand activity can be modulated by light, but also offers the potential of real-time on-target activity modulation of H_1R by light in living cells.

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Oral Presentations

O27

A HISTAMINE H_3 RECEPTOR (H_3R) ANTAGONIST-NITRIC OXIDE (NO) DONOR HYBRID COMPOUND IN THE PREVENTION OF POST-ISCHEMIC PHOTORECEPTOR DEGENERATION

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Ischemia is a pathological condition consisting in a restriction of blood flow to a specific organ. At ocular level, ischemia can depend by various diseases, such as diabetic retinopathy or glaucoma of which, it can be both a cause and a consequence.

There are different pharmacological approaches for the treatment of ocular ischemia, most of them are addressed to reduce the intraocular pressure (IOP) and to ameliorate the hemodynamic of the ophthalmic artery.

Several studies demonstrate that nitric oxide (NO) is involved in vasodilation, IOP homeostasis and modulation of ocular blood flow. A recent publication demonstrate that a histamine H_3 receptor (H_3R) antagonist can ameliorate ocular blood flow suggesting a role of this amine in controlling ocular vascular tone.

Based on this evidence, our project evaluaed the capability of a histamine H_3R antagonist-NO donor hybrid compound, ST-1989, to reduce the IOP, to ameliorate the hemodynamic of ophthalmic artery and to preserve the degeneration of photoreceptors induced by the ischemic damage, in a rabbit model of retinal ischemia/reperfusion (I/R).

The I/R model was carried-out on New Zealand White rabbits through repeated injections of ET-1, twice a week for 6 weeks. IOP measurements were performed with a Pneumotonometer at baseline and every week for six weeks. The hemodynamic was evaluated with an Eco Color Doppler and the Pourcelot Resistivity Index was measured. The electroretinogram was used to assess the retinal function through the evaluation of the response of photoreceptors to a light stimulus. The animals were treated with vehicle or with compound ST-1989 twice a day for four weeks.

Hybrid compound ST-1989 demonstrated to be effective in reducing IOP. Moreover, this compound was able to ameliorate the vascular tone and to prevent photoreceptors damage induced by ET-1 injections. In conclusion this hybrid compound is a promising therapeutic strategy for the prevention of post-ischemic photoreceptors degeneration.

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Oral Presentations

O28

HISTAMINE H_3 RECEPTOR ANTAGONISTS INFLUENCING Aβ OLIGOMERISATION

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Alzheimer's disease (AD) is the most prominent neurodegenerative disease in our time and requires a high level of pharmacological and medical research. The cause of AD is still unknown, but a loss of neurons and thereby the lack of neurotransmitters lead to impairments in memory and learning processes. Current symptomatic therapies focus on maintaining physiological conditions, e.g. through acetylcholinesterase inhibition. The amyloid hypothesis has been discussed for many years as an important component of the neuropathology of AD. Degradation products of the amyloid precursor protein, $Aβ_{1-40/42}$, accumulate into toxic oligomers or fibrils, which in the further course can accumulate into Aβ plaques leading to neuronal damage and cell death. Protein-protein interactions (PPI) play an important role in this context. Stabilisation of Aβ monomers is a promising approach to prevent oligomerisation. Here, we present multi-target directed ligands (MTDL) that act as PPI inhibitors on the one hand and as antagonists at the histamine H_3 receptor (H_3R) on the other. Inhibition of H_3R shows procognitive effects through its action as a heteroreceptor on non-histaminergic neurons, affecting various neurotransmitters including acetylcholine. The structural design of the synthesised compounds is based on the linkage of H_3R pharmacophore with peptidomimetic structures, mainly keto, diketo and acyl derivatives of piperazine. H_3R affinity was determined by radioligand displacement assays, while affinity to Aβ monomers was determined by surface plasmon resonance. A total of 16 compounds were synthesised, some of which showed low nanomolar affinity for H_3R, including two compounds with good affinity for Aβ monomers. One compound was identified as a new lead structure for further development of the presented compounds. The combination of procognitive H_3R affinity and Aß monomer affinity may be a promising MTDL approach to combine symptomatic with potential causal AD therapy.

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Oral Presentations

O29

DETECTION OF H3 AND H4 RECEPTOR mRNA EXPRESSION AT THE CELLULAR LEVEL

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H3 and H4 receptors (Hrh3, Hrh4) play significant physiological roles including gastroprotection. Both are also up-regulated in diabetic rat kidneys. The growing potential of Hrh3 and Hrh4 as novel therapeutic targets requires localization of them at the cellular level, which is essential for understanding their functional roles.

We used nonradioactive *in situ* hybridization assay with DIG-labeled antisense riboprobes to detect mRNA expression of Hrh3 and Hrh4 in mouse brain, stomach, kidney, and brown adipose tissue. DIG-labeled sense riboprobes were used as controls. For Hrh3 mRNA expression, tissues from $Hrh3^{-/-}$ mice also served as negative controls.

Hrh3 mRNA was detected in various brain areas with patterns similar to those obtained from radioactive *in situ* hybridization. Prominent expression was seen in the hippocampus (granule cells of dentate gyrus, and pyramidal neurons in CA1-3), habenula, thalamus, hypothalamus, while moderate to strong signals were detected in different cortical areas (most likely in pyramidal neurons) and olfactory bulb. Moreover, H3 receptor was detected in the kidney (glomeruli, Bowman's capsule and convoluted tubules), stomach (most abundant in cells of the basal gastric gland) and brown adipose tissue.

Hrh4 mRNA was detected in the kidney, stomach and brown adipose tissue, with fairly low expression intensity. In the kidney, sparse Hrh4 mRNA-positive cells were seen in convoluted tubules. In the stomach, H4 mRNA was detected in some gastric glandular cells and occasionally in mucous epithelial cells. In the brown adipose tissue, H4 receptor was expressed in brown adipocytes and the endothelial cells of blood vessels.

So far, this nonradioactive *in situ* hybridization method has allowed us to identify several types of cells expressing Hrh3 and Hrh4 in different tissues. This information and application of the method for experimental conditions will reveal cellular circuits in which the receptors are involved in different tissues.

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Oral Presentations

O30

PHARMACOLOGICAL CHARACTERIZATION OF PHOTOCAGED HISTAMINE H_3 AND H_4 RECEPTOR AGONISTS

Meichun Gao, Yang Zheng, Maikel Wijtmans, Henry F. Vischer and Rob Leurs

The photocage strategy enables precise, one-way spatio-temporal control of ligand bioactivity by removing a protective group using light to allow subsequent ligand interaction with the target of interest. The histamine H_3 and H_4 receptors are G protein-coupled receptors that are involved in cognitive-related processes and inflammatory responses, respectively. We have developed photocaged agonists for the H_3R and H_4R by protecting immepip and 4-methylhistamine, respectively, with a photo-responsive BODIPY group, resulting in a more than 10-fold decrease in receptor binding affinity. Illumination of BODIPY-caged immepip (VUF25657) compounds at 560 nm resulted in rapid uncaging within minutes as detected by LC-MS and restored binding affinity and efficacy in radioligand binding and a FRET-based cAMP biosensor. Light-induced uncaging of BODIPY-caged 4-methylhistamine (VUF25678) was confirmed by LC-MS analysis. However, low solubility in assay buffer prevented accurate assessment of the pharmacological parameters for VUF25678 and also prevents its application. In conclusion, BODIPY-based photocaging of histaminerigic agonists has proven to be an effective strategy to obtain a photocaged H_3 receptor agonist, that will serve as a new photosensitive GPCR tool for the spatio-temporal control of the H_3 receptor.

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Poster Presentations Abstracts

EHRS 2022 Hannover

Poster Presentations

P1

SIMULTANEOUS H3R/D2R/D3R ANTAGONISM MITIGATES AUTISM-LIKE BEHAVIOR IN MICE

Bassem Sadek, N. Eissa, K. Venkatachalam, S. Zhong, Holger Stark

Autistic spectrum disorder (ASD) is a neurodevelopmental brain disorder. Central dopamine and histamine have influence on behavior in brain disorders including Alzheimer's disease, schizophrenia, anxiety, and narcolepsy, all of which are comorbid with ASD.
The effects of systemic treatment with the novel multiple-active histamine H3 receptor (H3R) antagonist and dopamine D2/D3 receptor (D2R/D3R) antagonist ST713 with high H3R antagonist affinity and balanced inhibitory effects on both dopaminergic receptor subtypes D2R and D3R [3] on ASD-like repetitive and anxiety behaviors in male BTBR T+tf/J mice models of ASD were evaluated. ST-713 (2.5, 5, and 10 mg/kg, i.p.) mitigated repetitive self-grooming behaviors, and significantly ameliorated the aggressive behaviors of BTBR T+tf/J mice in social dominance test (all $P<0.05$). Moreover, ST-713 modulated disturbed anxiety/fear-like levels in elevated plat form task, but did not alter increased hyperactivity of the tested mice. Furthermore, ST-713 (5 mg/kg) attenuated the increased levels of hippocampal and cerebellar protein expressions of Tumor Necrosis Factor-alpha , Interleukins-1β , and IL-6 in treated BTBR T+tf/J mice brains (all $P<0.01$). The observed effects of ST-713 on self-grooming and aggressive behaviors were entirely reversed by co-administration of the H3R agonist (*R*)-α-methylhistamine or the anticholinergic drug scopolamine. These in vivo observations offer evidence for the potential therapeutic role of such multiple-targeting compounds in chronic neurodevelopmental disorders, such as ASD, albeit further in vivo investigations in different animal ASD models and various species are still warranted.

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Poster Presentations

P2

A HISTAMINERGIC SOURCE IN THE BED NUCLEUS OF STRIA TERMINALIS: IMPLICATIONS FOR STRIATAL PHYSIOLOGY AND NEURODEVELOPMENT

Marquez Gomez, R. and Ellender, T.

Tourette's syndrome (TS) is a neurodevelopmental disorder of largely unknown aetiology characterized by motor and vocal tics, and comorbidities such as anxiety and obsessive-compulsive disorder. Familial cases of TS have suggested a mutation in the histamine decarboxylase gene as one of the main genetic causes of TS which possibly affects the development of the brain's Social Decision-Making Network (SDMN) including basal ganglia and striatum, limbic system, and hypothalamus.

Using electrophysiological and optogenetic approaches in mice we studied the roles of histamine during early postnatal striatal development. We find evidence that direct histamine application modulates the intrinsic electrical properties of D1 and D2-expressing young striatal spiny projection neurons (SPNs), resulting in overall increases in their excitability, as well as inhibiting both prefrontal and visual cortex input onto SPNs. Contrasting with the pharmacological evidence, immunohistochemical staining of young mouse brain sections revealed only sparse or absent striatal innervation by histamine-containing fibres, making the striatal source of histamine contentious. Although striatum was largely devoid of histamine-containing fibres we observed that the neighbouring Bed Nucleus of Stria Terminalis (BNST) was heavily innervated at early postnatal ages. The BNST is a part of the limbic system and SDMN and involved in the appearance of anxiety-related behaviours. Given the low striatal histaminergic innervation at early postnatal stages and close apposition to striatum we lastly explored whether the BNST could serve as an extra-striatal source of histamine. Preliminary evidence suggests that histamine from the BNST can impact striatum and modulate cortico-striatal synaptic transmission.

Our results suggest that BNST can act as a paracrine source of striatal histamine and suggests a new synergy between striatum, histamine and BNST that can help us better understand the diversity of TS symptoms.

Poster Presentations

P3

NEUROPROTECTIVE VERSUS NEUROTOXIC PROPERTIES OF SALSOLINOL AND ITS ENANTIOMERS

M. Kurnik-Łucka, J. Goryl, N. Khan, M. Rivera, G. Latacz, K. Gil

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, SAL), since its first detection in the urine of Parkinsonian patients treated with L-DOPA, has been proposed as a possible neurotoxic contributor to the disease. While MPTP and its metabolite 1-methyl-4-phenylpyridinum ion (MPP^+) are well recognised dopaminergic neurotoxins. However, SAL might also possess neuroprotective properties due to the presence of catechol moiety. Previously, we confirmed its antioxidant and neuroprotective properties in vitro (lactate dehydrogenase test as well as MTS, ROS, and caspase activity assays). The aim of the present study was therefore to purify SAL enantiomers and to compare the neuroproptective properties of R-SAL, S-SAL and the racemate in vitro.

R,S-SAL was purified by means of HPLC with retention time 17.058 min and 21.575 min for S-SAL and R-SAL, respectively. SH-SY5Y cells were seeded at a concentration of 2.5×10^4 cells/well and cultured for 24 h to reach 70% confluence. Cells were preincubated for 1 h with either R,S-SAL or enantiomers and next either MPP+ (1000 µM) or H_2O_2 (350 µM) was added. After 24-48 h of incubation, the MTS assay was used to assess cell viability.

The eluates contained S-SAL with less than 0.1% of R-SAL as well as R-SAL with about 4% of S-SAL, were aliquoted, lyophilized, and stored in dark microtubes. The amount of the purified SAL enantiomers was further checked spectrophotometrically. SH-SY5Y cells' viability was indifferent between R,S-SAL and its enantiomers at the concentration of 50 µM. Cell viability was significantly increased in SH-SY5Y cells exposed to a mixture of R,S-SAL (50 µM) and MPP^+ (1000 µM) in comparison to MPP^+ alone as well as exposed to a mixture of R or S-SAL (50 µM) and H_2O_2 (350 µM) in comparison to H_2O_2 alone.

Our preliminary data suggest that possible neuroprotective role of SAL may not necessarily be related to stereoselectivity and confirm that R,S-SAL and its enantiomers are non-toxic at low doses.

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P4

IN VIVO PRELIMINARY EVALUATION OF DL123, A NEWLY SYNTHESIZED H_3 RECEPTOR ANTAGONIST, AND ITS THERAPEUTIC POTENTIAL IN PARKINSON'S DISEASE

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At present, the treatment of Parkinson's disease (PD) is based on the use of drugs to act on a single molecular target. Current pharmacological approaches are limited in their ability to modify significantly the course of the disease. New therapeutic strategies comprise drug designed to act on multiple neural and biochemical targets.

In the present study, we have focused on the effects of DL123, 1-(3-(4-(*tert*-butyl)phenoxy)propyl)-2-methylpyrrolidine, that combine two desirable properties, i.e. MAO B inhibition (IC_{50}=4 nM) and good affinity for human histamine H_3 receptor (K_i=25 nM), on cerebral catecholamines. It was previously confirmed that DL123 crosses the BBB, evokes typical effects on feed intake for H_3R antagonist and has low cytotoxicity.

The experiment was performed on Male Wistar. DL123 (3 mg/kg) was given sc. for 6 days. Pitolisant, (H_3R antagonist/inverse agonist) in the same dose was used as a reference drug. The concentration of dopamine (DA) and noradrenaline (NA) as well as their metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylglycol (MHPG) was determined in striatum (STR) and cerebral cortex (CTX) with RP-HPLC-ED method.

In DL123-treated group, a statistically significant increase in DA content in STR and CTX was noted, compared with the results obtained for control (STR: 9.91±1.72 *vs.* 5.24±0.62 nmol/g wet tissue, CTX: 1.78±0.13 *vs.* 1.13±0.15 nmol/g wet tissue). These results correspond with a decline in DOPAC/DA ratio in brain structures (STR: 0.21±0.07 *vs.* 0.51±0.04, CTX: 0.36±0.08 *vs.* 0.89±0.11). Additionally, DL123 administration also slightly rose NA concentration in CTX and statistically significant decreased MHPG concentration, expressed as lower MHPG/NA ratio (0.79±0.28 *vs.* 1.47±0.13).

In conclusion, the inhibition of MAO B activity, H_3R antagonistic properties and the ability to increase cerebral DA concentration predispose DL123 to further experimental studies to assess its therapeutic potential in PD.

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P5

EFFECTS OF HEALING EARTH (HE) IN VITRO AND IN VIVO WITH REGARD TO HISTAMINE (H) BINDING AND REDUCTION OF H-MEDIATED SYMPTOMS – A PROMISING APPROACH FOR H INTOLERANCE SYNDROME (HIS) AND IRRITABLE BOWEL SYNDROME (IBS)

J. Kleinhenz, K. Hotfiel, O. Cheremina, W. Najajrah, M. Radecki, M. Raithel

In allergy, IBS, MCAS the absorption of biogenic amines by the intestine play an important role. These patients often report H-mediated symptoms, are treated by antihistamines or avoiding certain foods. Naturally pure HE from glacial loess, has a large surface area, detoxifies radicals and has the potential to bind various substances such biogenic amines. Patients: In vitro approach, it was first examined whether and to what extent HE can bind H. Physical binding studies with 10 or 40 mg H on 20 g LUVOS HE were carried out for 1 and 2 h at 37 °.

The amount of H at time 0 or after 1 and 2 h was determined using the HPLC / DAD method. In a non-interventional study over 20 days, 27 pts. with known HIS ingested 3 x 6.5 g HE/d. A subjective scoring system, tolerance, side effects and pain score were recorded in order to determine a possible effectiveness of the HE through the binding of H.

Compared with controls (100% H recovery), 20 g HE could achieve a binding of approx. 35% or 48% of the H used <60 min. with the 10 or 40 mg H dose in vitro [Δ reduction 0.73- or 3.75mg/dl]. Further binding by longer incubation showed no additional effect.

Interestingly, 5 pts. (18.5%) completely lost their symptoms and had remission; 4 of them were able to do without the H-free diet.

7/27 pts. (25.9%) showed a very clear regression of their H symptoms (> 75%) compared to the score before therapy, 8 (29.6%) a good symptom reduction (50-75%). The remaining 7 pts. (26%) were unchanged by HE.

HE provided by Luvos showed a potentially therapeutic binding effect for H in vitro. As H concentrations are much lower in vivo, it is concluded that HE is responsible for luminal H binding in the patients. This effect was found beneficial to reduce H symptoms in more than two thirds of the pts. In view of increasing numbers of allergy, HIS and MCAS, HE can represent a potentially significant, non-immunosuppressive alternative therapeutic target for controlled prospective studies in this field.

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Poster Presentations

P6

HISTAMINE H_4 RECEPTOR EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype. There are neither universally accepted prognostic markers, nor molecular targets related to TNBC. The histamine H_4 receptor (H_4R) has been characterized in TNBC experimental models, demonstrating its critical role in tumor development and progression. In this study, the H_4R expression was compared in breast cancer subtypes and correlated with clinical features using The Cancer Genome Atlas data (TCGA). The H_4R status was further evaluated by immunohistochemistry in 30 TNBC human samples in relation to clinicopathological parameters. Analyses of TCGA Pan-Cancer Atlas data set show that H_4R mRNA expression was lower in the aggressive basal-like/TNBC tumors compared with the more favorable clinical outcome luminal A subtype (P=0.028). A high level of H_4R was significantly associated with improved relapse-free survival (HR 0.77, P=0.016) and overall survival (HR 0.64, P = 0.019) in basal-like cancer patients. To corroborate the bioinformatic analyses, we investigated the protein expression of H_4R in TNBC samples, according to pathology-based classification. The specificity of the antibody was checked using HEK293 cells, which do not endogenously express H_4R. Membranous and cytoplasmic H_4R immunostaining was detected in about 70% of TNBC samples, and its expression was positively correlated with the levels in the histologically normal peritumoral tissue. High H_4R expression in peritumoral tissue correlated with reduced number of lymph node involvement and unifocal TNBC while it was associated with increased patient survival. In conclusion, the H_4R might represent a potential prognostic biomarker in TNBC, and a promising therapeutic target for this aggressive and difficult-to-treat type of breast cancer. Further studies in large cohorts are needed to better understand the significance of H_4R in breast cancer biology.

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Poster Presentations

P7

PROGNOSTIC POTENTIAL OF HISTAMINE H_4 RECEPTOR EXPRESSION IN RENAL CANCER

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Renal cell carcinoma (RCC) is one of the most prevalent urological malignancies worldwide. Clear cell RCC (ccRCC) represent about 80% of RCC subtypes and show distinct immunological features and low 5-year survival rate. The expression of histamine H_4 receptor (H_4R) was linked to clinicopathological features and tumor grade in different types of cancer. However, there is no evidence of the protein expression of H_4R in RCC. The aim of this work is to first evaluate the H_4R expression in ccRCC using The Cancer Genome Atlas (TCGA) data and correlated it with clinical attributes. To validate the transcriptomic data, we next evaluated the H_4R protein expression by immunohistochemistry in 13 normal renal tissue and in 31 ccRCC samples. Mast cell count and tumor-infiltrating lymphocytes (TILs), including tumor-intraepithelial and stromal lymphocytes, were also analyzed.

ccRCC exhibited increased H_4R gene expression compared to normal tissue. Within tumor samples, G4 neoplasm histologic grade showed the lowest levels of H_4R gene expression. A high level of H_4R was significantly associated with increased disease-free survival (P=0.0466) in ccRCC patients.

Likewise, the H_4R protein expression levels were significantly higher in ccRCC specimens than in normal renal tissue (Mann-Whitney test, $P<0.0001$). Interestingly, H_4R expression varied with the histological tumor grading (ANOVA, $P<0.01$). Extreme nuclear pleomorphism grade 4 tumors show the highest percentage of stromal TILS and the lowest number of mast cells and H_4R levels compared with grade 2 and 3 ccRCC ($P<0.01$). In conclusion, this study improves the knowledge of the role of H_4R in ccRCC biology and could provide a venue for the development of a new diagnostic tool and/or therapeutic target.

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Poster Presentations

P8

H1 AND H3 RECEPTORS ARE INVOLVED IN THE SYNTHESIS IL-27

Khanferyan R.A.

Histamine is a well-known mediator of inflammatory reactions. Histamine can induce the synthesis and the secretion in PBMC cultures of the multiple cytokines, like IL-18, IL-6, gIFN e.c. via different receptors. Nevertheless, previously it have been demonstrated that histamine had no impact on the production of another proinflammatory IL-12, which can strongly influence on the differentiation of CD4+ T progenitors. IL-12 cytokine family includes an IL-27 structurally similar to proinflammatory IL-6. IL-27 consists of 2 subunits: IL-27B - known as Epstein-Barr virus (EBV)-induced gene 3 (EBI3) and IL27-p28 - known as IL-30. IL-27 is highly synergistic to IL-12 in activating an antigen-presenting cells and CD4+ T progenitors towards Th1 switching and□□□□□ production as well. The goal of this study was to investigate the possible role of histamine $H_1$and H_3 receptors in the synthesis of IL-27 cytokine. The concentration of IL-27 assayed in supernatants of 72-h cultivated PBMC of 7 healthy donors by immunofluorescence method on Luminex-200 equipment (Luminex Corporation, USA) using multiplex assay kit "ProcartaPlexTMH .Cyto-/Chemokine/Growth Factor Panel 1" (eBioscience, Austria). The study demonstrated very low spontaneous IL-27 synthesis by cultivated PBMC of healthy donors. The results had a wide scatter of individual data, but in all cultivated donor's PBMC it have been demonstrated very high increase in the secretion IL-27 ($p<0.05$) in the presence of H_3 receptor antagonist Ciproxifan (10^{-5}M/ml). Similar results have been shown in IL-27 synthesis after the activation of H_1 receptors by 2-Methylhistamine (10^{-5}M/ml), but an increase in the level of IL-27 was less pronounced ($p<0.05$). Thus, the study demonstrated an important role of H_1 and H_3 receptors in IL-27 synthesis. H_1 and H_3 histamine receptor active compounds may have an impact on the differentiation of CD4(+) T precursors via modulation of IL-27 synthesis. The activation of H_1 and H_3 receptors may have an opposite effect on the synthesis of IL-27.

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Poster Presentations

P9

HISTAMINE H_3/H_4 RECEPTORS AND THE SYNTHESIS OF GROWTH AND NEUROTROPHIC FACTORS BY MONONUCLEAR AND DENDRITIC CELLS

Khanferyan R.A.

Histamine and its receptors (H_1R–H_4R) expressed in many cells, including mast cells, mononuclear, dendritic and other cells play a crucial and significant role not only in the development of various allergic. Histamine is a pleotropic factor playing a crucial role in non-allergic processes. It is also important factor for homeostatic regulation of energy levels, sleep-wake cycle, cognition, inflammation, synthesis of various cytokines, chemokines and growth factors. The goal of the study was to compare the synthesis of multiple growth and neurotrophic factors (epidermal growth factor-EGF, hepatocyte growth factor-HGF, platelet-derived growth factor-PDGF-BB, vascular endothelial growth factors-VEGF-A and VEGF-D, basic fibroblast growth factor-FGF-2, interferon-induced protein-IP-10, placental growth factor-PIGF-1, brain-derived neurotrophic factor-BDNF and nerve growth factor-NGF - β) by mononuclear cells (PBMC) and dendritic cells (DC) derived from PBMC. The concentration of growth and neurotrophic factors have been assayed in supernatants of PBMC and DC of healthy donors (n-6) cultivated during 48h by Luminex xMAP technology using 45-plex Human Cytokine/Chemokine/Growth Factor Elisa Kit (eBioscience, Procarta). Cells were cultivated in the presence of H_3/H_4 antagonist Ciproxifan (10^{-5}M/ml). In our preliminary study it have been demonstrated that histamine have an impact on the synthesis of the most of growth factors by PBMC and DC. In this study we demonstrated the marked differences in the production of the growth and neurotrophic factors by PBMC and DC. The concentration of EGF, VEGF, BDNF and β -NGF were much lower in the supernatants of cultivated DC than in PBMC. On the other hand, the synthesis of HGF, PDGF-BB, IP-10 and PIGF was upregulated in cultivated DC. The analysis of the role of histamine receptors in the regulation of the growth factors synthesis by DC showed that H_3/H_4 antagonist Ciproxifan decreases the production of the most of the factors: VEGF-A (in 1.17 times), IP-10 (1.64), BDNF (4.02) and b-NGF (2.72). Only the production of EGF and PIGF increases in 2.85 and 1.28 times, respectively. It have been demonstrated no or very low effect of Ciproxifan on the synthesis of PDGF-BB and PIGF-1. Thus, the synthesis of growth and neurotrophic factors by PBMC and DC derived from PBMC differ significantly. Histamine and histamine H_3/H_4 receptors may have an impact on the synthesis of growth as well as neurotrophic factors by PBMC and DC.

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P10

UNDERSTANDING THE IMMUNOMODULATORY ROLE OF HISTAMINE H_4 RECEPTOR IN BREAST CANCER. PERSPECTIVES TOWARDS AN IMMUNOTHERAPEUTIC APPROACH*M.B. Nicoud, I. Ospital, M.A. Táquez Delgado, D. Speisky, A Vidal, G.A. Cremaschi, H. A. Sterle, Vanina A. Medina*

The histamine H_4 receptor (H_4R) is a potential therapeutic target for inflammatory and autoimmune diseases. Recently, we have reported the participation of the H_4R in the antitumour immunity in breast cancer. This study aimed at further exploring the role of H_4R in the immunobiology of breast cancer.

We developed a syngeneic breast cancer mouse model by orthotopic inoculation of 4T1 breast cancer cells in H_4R knockout (H_4R-KO) and wild-type (WT) mice. Treatments (mg kg-1): histamine (5), H_4R agonist JNJ28610244 (1 and 5), and H_4R antagonist JNJ7777120 (10).

Histamine reduced tumor growth and increased tumor apoptosis, effects that were significantly enhanced in H_4R-KO mice. Histamine reduced the percentage of tumor-infiltrating T helper (CD4+) cells and myeloid derived suppressor cells (MDSC), and increased the NK cells only in H_4R-KO mice. Significant Spearman's correlations between tumor weight and cytotoxic lymphocyte infiltration were detected only in histamine-treated H_4R-KO mice (NK cells, r:-0.6364; CD8+ cells: r:-0.7697, $P<0.05$) but not in WT mice. The therapeutic and immunomodulatory effects of the H_4R agonist depend on the administered dose. The lowest dose slightly reduced tumor size and increased the percentage of CD4+ T cells in the tumor-draining lymph node (TDLN). The highest dose produced an immunosuppressive effect on the TME, increasing CD4+CD25+FoxP3+ Treg cells and IL-10 levels, while reducing IFNγ ($P<0.05$). In this line, the H_4R antagonist reduced the proportion of tumor-infiltrating CD4+ T cells and Tregs in the TDLN. In vitro cell viability assays showed that histamine, acting directly through the H_4R on 4T1 tumor cells, reduced cell proliferation.

We conclude that histamine produces a complex regulation of breast cancer biology, producing direct effects on cancer via tumor cell-intrinsic pathways, and contributing to the modulation of the tumor microenvironment by controlling immune-mediated effects.

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P11

SYNTHESIS AND EVALUATION OF ARYL-ALKYLPIPERAZINE COMPOUNDS AS DUAL LIGANDS AT H_3R AND CHOLINESTERASES

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The noteworthy involvement of histamine receptors on cognition has attracted interest of many researchers. The histamine H_3 receptor (H_3R) is an auto and hetero-receptor that may control the release of several neurotransmitters besides histamine; thus H_3R antagonists may increase the release of acetylcholine (ACh), also involved in the cognitive processes. Therefore, the mutual action on pharmacological targets capable of increasing the activity of both histamine and ACh may represent an important alternative in the treatment of dementias. With this regard, a set of aryl-alkylpiperazine derivatives were synthesized and evaluated as H_3R/cholinesterases ligands. The structural motifs required for binding at H_3R were overlapped to anticholinesterase agents and used as model to design the compounds. The amide- and carbamide-containing compounds were synthesized with yields up to 67%. These compounds were tested for the ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) at 1 and 100 µM and the most promising were further evaluated in full concentration-response curves, that allowed estimating the IC_{50} values. The affinity to the H_3R was assessed through binding assays and the K_i values were then calculated. The compounds showed low affinity at both cholinesterases, although two compounds showed measurable inhibition at AChE. On the other hand, six compounds showed considerable affinity at the H_3R, with compound LINS05a123 being highlighted due to its submicromolar affinity (pK_i = 6.27). This compound also showed selective inhibitory activity on AChE (IC_{50} = 198 µM). Compound LINS05c214 is also highlighted due its higher inhibitory potency at AChE (IC_{50} = 112 µM) and considerable affinity at the H_3R (pK_i 5.83), being the most interesting by considering the multitarget proposal. The results suggest that longer linker groups along with pyridylpiperazine as basic motif contributes to the observed activity at both targets. The data obtained provide important information that will help in the development of further multitargeted compounds.

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P12

ERGOTAMINE INCREASES FORCE OF CONTRACTION IN ISOLATED HUMAN ATRIUM VIA H_2-HISTAMINE RECEPTORS

Gergs U, Schwarz R, Azatsian, K, Höhm C, Kirchhefer U, Hofmann B, Neumann J

We wanted to know whether a derivative of lysergic acid namely ergotamine acts an agonist at cardiac human H_2-histamine receptors or at cardiac human 5-HT_4-serotonin receptors. At least in binding data ergotamine sometimes used in migraine patients was of nanomolar affinity for 5-HT_4-receptors in vitro (Headache, 2003; 43: 144). For ergotamine at H_2-receptors hardly any binding was measureable. Nevertheless, we measured the effects of ergotamine on the force of contraction in isolated electrically stimulated (1 Hz) left atrial preparations (LA), spontaneously beating right atrial preparations (RA) from H_2-histamine- or 5-HT_4-receptor-overexpressing hearts from transgenic mice (H_2-TG, 5-HT_4-TG), wild type mice (WT) and isolated human atrial preparations from bypass patients (HAP). Ergotamine alone exerted concentration- and time-dependent positive inotropic (LA) and positive chronotropic (RA) effects starting at 0.1 µM and reaching a plateau at 1 µM in H_2-TG and 5-HT_4-TG but not in WT. The positive inotropic effects of ergotamine in LA and A from H_2-TG or 5-HT_4-TG could be antagonized by 10 µM cimetidine or tropisetron, respectively. Ergotamine (1, µM, 3 µM and 10 µM) alone failed to increase force of contraction in HAP, but was effective in the are pre-incubation with 1 µM cilostamide, which effect was antagonized by 10 µM cimetidine ($n=5$, $p<0.05$) but not by tropisetron. In summary, we present evidence that ergotamine is an agonist at cardiac human H_2-histamine receptors.

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P13

EFFECT OF TEMPERATURE ON CONTRACTILITY OF ISOLATED H_2-RECEPTOR OVEREXPRESSING MOUSE ATRIAL PREPARATIONS

Hoffmann R, Gergs U, Kirchhefer U, Neumann J

Histamine can induce positive inotropic and chronotropic effects in the human heart via H_2-receptors. It is conceivable that the H_2-receptors alter their function in fever (hyperthermia) or in patients with artificially lowered temperature (hypothermia) used sometimes in surgery. To address this issue, we used transgenic mice (H_2-TG) with cardiomyocyte-specific overexpression of the human H_2-receptor and, for control, wild-type mice (WT) that lack a functional H_2-receptor. Hypothermia or hyperthermia were achieved by lowering or raising the temperature of the organ bath from 37°C to 23°C or 42°C. In isolated left or right atrial preparations, histamine was cumulatively applied.

Histamine induced positive inotropic and chronotropic effects only in atria of H_2-TG but not of WT, whereas isoprenaline was effective in both, WT and H_2-TG. The beating rate was always lower in WT than at H_2-TG, and in both, WT and H_2-TG, lower at 23°C (WT=57 ±7, H_2-TG=120 ±11, n=15-16, $p<0.05$ vs. WT) and higher at 42 °C (WT=419 ±24, H_2-TG=529 ±36, n=9-13, $p<0.05$ vs. WT) than at 37°C (WT= 359 ±19, H_2-TG 453 ±24, n= 15-16, $p<0.05$ vs. WT). The potencies of histamine on beating rate in H_2-TG were not significantly different (37°C: EC_{50}=7.1±0.1, 23°C: EC_{50}=7.2±0.1, 42°C: EC_{50}=7.1±0.2, $p>0.05$). The potency of histamine to raise force of contraction in left atrial preparations was lower at 23°C (EC_{50}=6.7±0.1, n=15) and at 42°C (EC_{50}=6.6 ±0.1, n=15) than at 37°C (EC_{50}=-7.0 ±0.1, n=15, $p<0.05$). We conclude that the contractile function of the human H_2-receptor is temperature dependent.

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P14

SEARCH FOR CHOLINESTERASE INHIBITORS AMONG NOVEL HISTAMINE H_3 RECEPTOR LIGANDS - BENZOPHENONE DERIVATIVES

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The loss of cognitive function in Alzheimer's disease is connected with the damage of cholinergic transduction in the brain and the lowering of acetylcholine levels. Enhancement of cholinergic neurotransmission in the brain can be achieved by blockade of histamine H_3 receptor (H_3R) or through the combination of H_3R antagonism with e.g. acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE) inhibition. We described previously xanthones as the promising multidirectional ligands. In continuation of this work, we developed benzophenone derivatives that are structural analogues of xanthones. A series of compounds was designed, synthesized and biologically evaluated.

The affinity of compounds at human H_3R was tested in a binding assay using [^{3}H]N^{α}-methylhistamine as radioligand in HEK293 cells stably expressing human H_3R. Cholinesterase inhibitory activities were evaluated by spectrophotometrical Ellman's method using AChE from electric eel and BuChE from horse serum. Moreover, *in silico* and *in vitro* methods were used to evaluate the metabolism of the selected compound and neuroprotection in the SH-SY5Y neuroblastoma cell line against H_2O_2 (300 µM). The cells necrosis and viability were detected by LDH and MTS assays, respectively.

All ligands tested had good affinities at human H_3R in the nanomolar range (8 nM ≤ K_i < 400 nM), whereas not all showed inhibitory activity in the submicromolar/micromolar range for both cholinesterases (IC_{50} < 8 µM). The most promising compound was (4-fluorophenyl)(4-((5-(piperidin-1-yl)pentyl)oxy)phenyl)methanone (**E325**) with the highest H_3R affinity (K_i = 8 nM) and butyrylcholinesterase (BuChE) inhibitory activity (IC_{50} = 172 nM). Further studies with **E325** showed its moderate metabolic stability and weak neuroprotective activity.

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P15

EXPRESSION OF HISTAMINE RECEPTORS IN INTESTINAL CACO-2 AND NEURONAL P19 CELLS AND THEIR PHARMACOLOGICAL IMPACT ON EVALUATION OF ANTIHISTAMINIC DRUGS

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The aim of this work was to characterize the *in vitro* response to histamine of cultured neurons derived from mouse P19 embryonal carcinoma cells and how vegetal diamine oxidase (vDAO) and catalase modulate this response. It was also investigated to which extent vDAO may control Ca2+ mobilization in comparison to desloratadine, an antihistamine drug.

The viability of the P19 cells following treatments with vDAO and catalase was measured by the neutral red (NR) assay. Expression of histamine receptors H1 and H2 was identified by RT-qPCR. Considering the damaging effect of high intestinal level of histamine, this study was conducted first on intestinal Caco-2 cells using Fluo-4 AM probe as Ca2+ tracker. Confocal microscopy showed that vDAO down-regulated histamine-induced Ca2+ mobilization. This effect was stronger than that obtained with the synthetic desloratadine drug.

At a fixed concentration of histamine, sufficient to trigger Ca2+ mobilization (monitored by the fluorescent probe), vDAO at a concentration three times lower than that of desloratadine, was similarly effective at inhibiting the same levels of Ca2+ flow. The exposure of P19 neurons to 0.1-2 mM histamine reduced their viability to 65%. This effect appeared to involve histamine receptors since it was prevented by treatment with desloratadine and cimetidine, which are H1 and H2 antagonists, respectively. The expression of functional H1 and H2 receptors in P19 neurons was confirmed by RT-qPCR analysis. The H1 and H2 antagonists, as well as vDAO and catalase, counteracted the viability loss triggered by histamine in cultured P19 neurons.

In conclusion, it was found that Histamine-degrading vDAO can efficiently counteract the histamine-induced Ca2+ release at concentrations lower than those of desloratadine, which competes with histamine. The neurones derived from murine P19 embryonal carcinoma cells appear as a convenient model to evaluate the action of antihistamine drugs.

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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

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
CHORUS

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

EHRS International Anthem

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

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.
CHORUS

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

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 eightytwo / 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.

EHRS International Anthem

CHORUS

13. And in nineteen eightyfour / 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.

16. And in nineteen eight 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.

CHORUS

17. Then to Marburg we returned/ ninetyone and also learned
That histamine in surgery´s no 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.
19. Then with Igor ninetyfive / and the Volga was alive
And we entered the Russian Golden Ring.
In Antwerpen ninetysix / Frans did show us a few tricks,
Singing Histaminosis all the day.

EHRS International Anthem

20. To Seville, once again / we all met in lovely Spain,
Singing Histaminosis all the day.
To Agnieszka ninetyeight / back in Poland it was great,
Singing Histaminosis all the day.
CHORUS

21. Then to Lyon ninetynine / 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.

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. Then to Bled we return / and once again could 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.

EHRS International Anthem

And to Durham we went then / in the year two thousand ten.
There with Paul near Cathedral did we stay.

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

28. Then to Łódź again next year / for the fourth time we meet here!!
Dear Agnieszka both Honorar and chair.
In two thousand and fourteen / Then Lyon was back on scene
And our Lin made it most amazing there.
CHORUS

29. Then to Málaga again / where it´s sun and never rain
And with Kika this was a lovely stay
In two thousand and sixteen / Florence once again was seen
In a gorgeous Emanuela way.

30. Then from Rob we got a call / Amsterdam invited all
Coming back to the sparkling Tulip Land
In two Thousand and eighteen / Lovely Dublin was the scene
There with Astrid a meeting really grand.

31. Back to Poland the next year / we for Katarzyna cheer
Splendid chemist in a lovely Krakow town.
The Pandemic came along/ with no meetings and no song
But then Histamine will always be are own

32. Then in twentytwentytwo/ Back to Hannover we flew
Celebrating the splendid 50 Years!
Let us sing this song together / Histamine will last forever
Singing Histaminosis all the way
CHORUS

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