Abstract Book

TURIN 2024

1st Joint Meeting of EHRS-HRSE

51st Meeting of the EHRS | 1st Meeting of the HRSE

23/25 May 2024

Turin, Italy

eventually meetings & events

PATRONAGE

DSTF DIPARTIMENTO DI SCIENZA E TECNOLOGIA DEL FARMACO Università degli Studi di Torino	DSTF – Dipartimento di Scienza e Tecnologia del Farmaco
UNIVERSITÀ DI TORINO	Università degli Studi di Torino
AdM Accademia di Medicina di Torino	Accademia di Medicina di Torino
SIF SOCIETÀ ITALIANA DI FARMACOLOGIA	Società Italiana di Farmacologia

SPONSOR

DSTF DIPARTIMENTO DI SCIENZA E TECNOLOGIA DEL FARMACO Università degli Studi di Torino	DSTF – Dipartimento di Scienza e Tecnologia del Farmaco
CliniSciences	CliniSciences S.r.l.

WELCOME MESSAGE

It is our great pleasure to welcome you to the 1st Joint meeting of the European Histamine Research Society (EHRS) and the Histamine Research Society Europe (HRSE) May 23-25, 2024 in Turin. This meeting represents a milestone for these two histamine research society: it is the 51th Meeting of the EHRS, and the 1st Meeting of the just born HRSE. Following the footsteps of the 50 preceding EHRS conferences the meeting will serve as a forum for the worldwide exchange of novel research results and ideas in the field of histamine and will provide an excellent opportunity for establishing research cooperation between leading international scientists from academia. The wide-ranged topics will be presented in 3 lectures including the Opening and GB West Lecture, 25 oral communications and 16 flash presentation (definitively replacing posters) and a round table on pharmacovigilance will offer the opportunity to contextualize the results from basic discovery into their clinical applications in function of the risk-benefit ratio. A session dedicated to the memory of Professor Pier Francesco Mannaioni will be held. Young Investigator Awards and best flash presentations competition will also take place.

Host this special meeting is a great honor for us, being the first time for Histamine Research delegates to meet in Turin. The city is a unique location at the base of the Alps in the northwestern corner of Italy. It was the first capital of Italy (1861 – 1865). The venue of the meeting, the *Accademia di Medicina di Torino*, is an example on how Turin has always represented a starting point for innovation in various sectors, including medical sciences. Indeed, in over 600 years of history, Turin's University is proud of its achievements, including the four Nobel Prize in Physiology or Medicine awarded to Camillo Golgi, Salvador Luria, Renato Dulbecco, and Rita Levi Montalcini**.**

Beside the science, we hope you will enjoy the charm of Turin in your free time. There are plenty of things to do in Turin. The city is well known for its baroque architecture and stately art-nouveau cafes. The Egyptian Museum is one of the biggest tourist attractions in Turin and showcases the largest collection of Egyptian artifacts outside of Cairo. The Mole Antonelliana is an incredible building, visible from all over Turin it has become the landmark of the city. It was initially designed as a synagogue but was later updated as a monument to national unity. You can take the lift up to the 85m platform for views of the city. Just next to it, you'll find the National Cinema Museum. If you're a petrol-head, definitely find time to visit Turin's famous automobile museum. It has a collection of around 200 cars from 80 different car brands! But do not forget the several Royal residences, From Palazzo Reale to Venaria Reale, and much more.

Please also take time to taste our food: you can taste incredible food and drink including truffles, delicious artisan cheeses, different varieties of rice, Piedmontese hazelnuts, and of course Barolo! Not to forget chocolate: Turin is, with the iconic Gianduiotto, Italy's chocolate capital. Since 2003, Turin organizes the much-loved festival, CioccolaTò. Do not also forget that the legendary Nutella was created in Piedmont by Pietro Ferrero in 1946. Finally for a real taste of Turin, do not forget to taste Bicerin, a combination of espresso, chocolate, and cream/whole milk.

We warmly welcome you to the 1st Joint meeting of the EHRS and HRSE and wish you all an exciting and highly rewarding meeting and pleasant stay in Turin.

On behalf of the Organizing Committee

Arianna Carolina Rosa

Chair of the Organizing Committee

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 Lion

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

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

2020's
2020 Online Symposium
2021 Virtual Event
2022 Hannover

VENUE

Accademia di Medicina di Torino - Via Po 18 10123 Turin, Italy

How to reach the Venue Google Maps: https:/GoogleMaps

Once you cross the entrance on Via Po 18 please **be brave...the building is still waiting the already planned renovation** but if **you go up the stairs on your left** on the landing you could admire the seventeenth-century fresco "La Crocifissione", attributed to Bartolomeo Guidobono (just restored and dedicated to the 22 Piedmontese doctors who sacrificed themselves during the COVID-19 pandemic), and **when you reach the first floor you will find yourself catapulted into a piece of history of Turin and medical sciences at the end of the corridor on you left**!

The *Accademia di Medicina di Torino*, was born from the initiative of nineteen doctors (including, Lorenzo Martini, Sacchetti, Pasero, Buosa, Averardi, Schina, Moris, Ricci, Griffa, Gallo, Bertini, Barovero, Riberi, Barbaroux) which in December 1819, in the midst of the Restoration, signed a request to meet periodically to debate medical scientific topics and to publish the reports of these sessions. Granted the authorization in February 1821, the first meetings could take place only in a strictly private manner at the private home of del Prof. Gallo, no possibility of public meetings was allowed. The political situation of the Savoy state reflected the revolutionary Italian context and the Austrian reaction. The public activity of the *Accademia delle Scienze* had been suspended; lessons at the University, already suspended for a certain time for fear of possible student demonstrations, had just been resumed. In 1832, the members of the Private Medical-Surgical Society addressed a request to the Sovereign to obtain funding for a magazine exclusively dedicated to medical topics on the work of the Medical-Surgical Society, today *Accademia di Medicina di Torino*. Four years later, in 1836, the first issue of the "Giornale delle Scienze Mediche", the official organ of the Medical-Surgical Society, published in Turin by the bookseller Reviglio, finally was published. In 1838 there were 29 members and became 40 in 1848. in 1842, the Society obtained recognition by the State which provided two rooms at the ground floor of Via Po 18, and guaranteed subsidization of publication costs and management activities and above all the possibility of holding four public meetings a year in the presence of a

police delegate in charge of control. The availability of the two rooms in Via Po 18 allowed to seat a public library.

On 10 February 1846, by royal decree signed Carlo Alberto, the Society was elevated to the rank of Royal Medical-Surgical Academy - *Reale Accademia di Medicina*. It has carried out its scientific and editorial activity uninterruptedly until today.

According to the declared objective of the association to spread out cultural themes and scientific renewal for the improvement of the Piedmontese medical class, the Academy Journal summarizes the entire history of Piedmontese medicine from the early nineteenth century to the present day. The Academy had also a key role also in the formation of the Faculty of Medicine starting from 1848, when the new chairs of Pathological Anatomy, Comparative Anatomy, Clinical Ophthalmology, Clinical of Mental Diseases, History of Medicine and General Pathology were settled. In 1865, when the capital was moved to Florence, the library collection was moved to the south wing of Palazzo Madama, where the Senate library was located. In those years, thanks to donations from private individuals and publishers, the number of conserved volumes reached 90.000. The library came definitively back to via Po 18, on the first floor, in 1891. The collection reached 100.000 units and was accessible to the medical public and students of the faculty of Medicine, including also a section dedicated to health legislation and public health publications. During the bombing of Turin in July 1943 the library, was completely burned down and about a thousand texts were recovered. Currently the library collection includes 11.761 books and monographs catalogued, 9.820 modern monographs (from 1831 to today), 1.941 old books (before 1830), 1.007 periodicals.



Nowadays the Academy of Medicine, guided by the President Prof. Giancarlo Isaia, has still a pivotal role in cultural initiatives for the training and updating of young doctors; with almost 100 Members it organizes periodical scientific sessions opened not only to the Members, but to the public.

GENERAL INFORMATION

Registration Desk

The registration desk is in the venue, at the first floor of Via Po 18 entered the *Accademia di Medicina di Torino*. **Please notice that no abstract book will be provided**, but for any convenience you can print or save the pdf file. **The certificate of attendance will be sent by email** at the end of the meeting. If you need a printed copy, please inform the secretary when you complete the check-in, so we can provide. If you still need to pay the Affiliation fees to the Society, you can do it at the EHRS desk. For any question during the meeting, you will find assistance at the Registration Desk, according to the Opening hours:
Thursday 09:30 – 17:00 h
Friday 08:30 – 17:30 h
Saturday 08:30 – 12:00 h

WiFi Connection

The wifi connection is guaranteed by the Eduroam (Education Roaming) service (https://eduroam.org/about/connect-yourself/).
Your password for your online identity is provided to you by your 'home' institution - where you are enrolled in study or are employed.
If for any reason you cannot use the Eduroam service and you need a wifi connection, we could activate a temporary Guest account to unito-guest wifi only if you required it by email at ariannacarolinarosaEHRS2024@gmail.com NOT LATER THAN the 20th of May. The temporary account is strictly personal, and you MUST have with you your Passport or Identity Card.

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.
The file of your presentation should be named as follows:
'PresentationTypeSessionNumber_Presenting Author.pptx', e.g., '**O1_Mustermann.pptx'.**

Young Investigators Award

The finalists will present their work in the form of a 15-minute oral presentation (10-minute presentation + 5-minute discussion) during the dedicated session on the 24th at 14.00.
The first and second-prize winners will be announced at the Gala Dinner, when all the finalists will receive a certificate and a prize. Moreover, the first and second-prize winners will be invited to publish their papers in Inflammation Research, following the normal reviewing process and payment of the fees if Open Access is desired. In the cover letter, it should be indicated that Professor Bernhard Gibbs should be the editor in charge as the EHRS-appointed editor. However, if another journal is more suitable for the work, an acknowledgment that the work was awarded with a Young Investigators Award must be included.

Flash Presentations

Oral presentations are scheduled not to extend 5 min, which includes 2 min 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.
The file of your presentation should be named as follows:
'PresentationTypeSessionNumber_Presenting Author.pptx', e.g., '**FP1_Mustermann.pptx'.**
A Flash Presentation Award Panel will review all eligible Flash presentation (the Presenter should be registerd as student) and **Flash Presentations Prizes** will be announced at the Gala Dinner and will receive a certificate and a prize.

Local Organizing Committee

Arianna Carolina Rosa (Torino, IT); Katerina Tiligada (Athens, EL); Ilona Obara (Newcastle, UK); Elisa Benetti (Torino, IT); Valentina Boscaro (Torino, IT); Margherita Gallicchio (Torino, IT); Simona Spampinato (Torino, IT)

Scientific Committee

Pierre Chatelain (BE); Madeleine Ennis (UK); Bernhard Gibbs (DE); Rob Leurs (NL); Detlef Neumann (DE); Ilona Obara (UK); Arianna Carolina Rosa (IT); Bassem Sadek (UAE); Holger Stark (DE); Satoshi Tanaka (JP); Katerina Tiligada (EL)

Organizing Secretariat

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Abstract Evaluation & Student Bursary Award Committee

Arianna Carolina Rosa (IT); Bassem Sadek (UAE); Satoshi Tanaka (JP); Katerina Tiligada (EL); Madeleine Ennis (UK)

Young Investigators Award (YIA) Panel

Bernhard Gibbs (UK), Beatrice Passani (IT)

Flash Presentations Award Panel

Detlef Neumann (DE), Satoshi Tanaka (JP), Katarzyna Popiolek-Barczyk (PL)

Publication of the meeting's proceedings

The proceedings of the meeting will be published in the journal Inflammation Research. All papers will be published as abstract and should confirm to the style of the journal printed pages.

SOCIAL PROGRAMME

Guided City Centre Walk and Welcome reception

On Thursday the 23rd at 17:30 we will start a **Guided City tour** across the most stunning and important squares of Turin.

We will discover the most iconic places of the unification of Italy walking through the small *Piazza Carlo Alberto* and *Piazza Carignano*.

We will admire the charming *Piazza San Carlo*, a little piece of Paris in Italy and finally we will walk through the royal Piazza Castello.

As a cherry on a cake, the roman area will give an archaeological ending to our journey just in time to reach COMBO Torino, Corso Regina Margherita 128, for the **Welcome reception.**

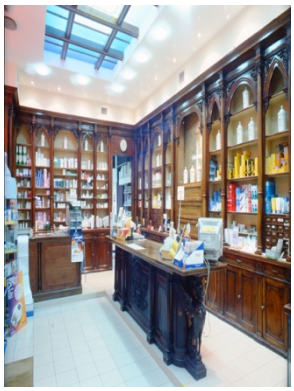
COMBO Torino is a dynamic, youthful, and eclectic space obtained from the renovation of a historic fire station in the multiethnic neighborhood of Porta Palazzo.

Porta Palazzo belongs to the historic centre of the city, yet it preserves a character of its own (the urban shape, the history, the settled people, the economic activities) which connotes it as a "popular" and multiethnic district. Porta Palazzo owes its name to one of the gates of the city, the old *postierla*, San Michele, which connected the suburban districts with the market located in what once was called piazza delle Erbe, now piazza Palazzo di Città. Throughout the centuries the postierla was replaced with a stone gate, and in the 17th century it took over the Porte Palatine as the main northern access in a definite way. Porta Palazzo was opened by 1701, under King Vittorio Amedeo II, but the first plan of piazza della Repubblica, however, was conceived within the so called "old city's" city plan reform, according to a project by architect Filippo Juvarra. Since the 19th century, efforts to embellish and shape the square have been manifold and the square's contours were completed in 1830.

As soon as the Juvarra exedra was completed, a spontaneous market sprung up, because of its favourable location in correspondence to the Milan axis, but the markets in Porta Palazzo settled once and for all in 29th August 1835, after a "Vicarial Manifesto" which, due to a cholera epidemic, prohibited trade in the piazzas of Palazzo di Città (once piazza delle Erbe) and Corpus. Today Porta Palazzo is the largest open-air market in Europe: it extends over a 51.300 sq m. area, houses a market which has a sale surface equal to 4.991 sq m. and over a thousand street vendors. The whole market area is visited by over 100.000 people, weekly.

Particularly fascinating the covered food market called "mercato dell'Orologio", built in 1916, and the Galleria Umberto I.

The latter originally was the Palazzo dei Cavalieri, which housed the Mauriziano Hospital from 1575 until 1884, and where is located the ancient Mauriziano Pharmacy from 1575.



Closed to the Porta Palazzo market, the historical flea market called "Balon" takes place. Since 1856, all Saturdays in a small version and every 2nd Sunday in the largest and famous version "Gran Balon", it is the perfect destination for enthusiasts and collectors in search of trouvaille or for young students or tourists in search of various curiosities.

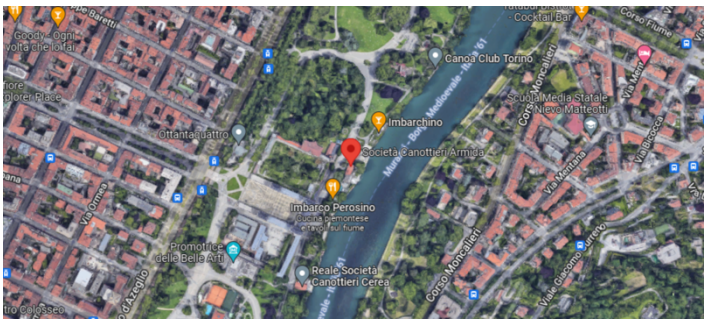
Starting from the 1960s Porta Palazzo become a point of reference for the many Italian immigrants who reached Turin in search of work. On Sundays, for example, it was the place where workers were sought for construction, but also where one could attend performances by improvised street artists.

The particular social context, the immense market and the urban and social degradation connected to them greatly encouraged the presence of voluntary associations and social commitment, such as the opening of SERMIG, also known as Arsenal of Peace on May the 24th 1964 by Ernesto Olivero.

In the 1980s, Italian immigration from southern Italy was completely replaced by people of African and Asian origin. Starting from 1996, the entire Porta Palazzo was the subject of a redevelopment project which led to a new road system and the recovery of some Juvarra buildings, but never lose the acquired "multiethnic skin". in March 2006, the lighting ceremony of the Paralympic Flame took place.

Gala Dinner

On Friday May 24, 2024 at 20:00 h we have our traditional Gala Dinner at the *Società Canottieri Armida* di Torino, Viale Virgilio 45, in the Parco del Valentino



Founded in 1869, the *Canottieri Armida* contributed in 1888 with other rowing clubs to the establishment of the Italian Rowing Club, nowadays known as the Italian Rowing Federation. One year later the Armida Rowing Club introduced one of the best rowing boats designed for four persons at the time, the Savoia.

In 1967 the Armida Rowing Club was awarded with the Golden Star for sporting merits by the Italian National Olympic Committee (CONI).

In 2006 the Armida Rowing Club resumed the ancient tradition of Venetian rowing, organizing courses and events to promote it. The venue offers a meeting place from which it is possible to enjoy peacefully one of the most suggestive sceneries of Turin for 140 years.

The main building, which was once the home of the "Pavilion of French Colonies" during the World Exhibition of 1911, is today supervised by the government department responsible for the environment and historical buildings.

INVITED SPEAKERS

PLENARY LECTURE - **Histamine and other biogenic amines in food: from scombroid poisoning to histamine intolerance**
Dr. Oriol Comas-Basté – University of Barcelona, Barcelona (Spain)

GBW LECTURE - **Mechanisms of mast cell inflammatory mediator secretion: an update**
Prof. Ulrich Blank – Institut National de la Santé et de la Recherche Médicale (INSERM) – Paris (France)

KEYNOTE LECTURE - **25 years of H4 receptor research**
Prof. Katerina Tiligada – University of Athens – Athens (Greece)

ROUND TABLE Anti-histamines in the real-world: pharmacovigilance in the spotlight
H_1-receptor affinity of psychotropics and risk of weight gain and metabolic disorders
Prof. Virginio Salvi – Policlinico Gemelli, Università Cattolica del Sacro Cuore – Roma (Italy)

ROUND TABLE Anti-histamines in the real-world: pharmacovigilance in the spotlight
Where has rantidine gone?
Dr. Marco Tuccori – Azienda Ospedaliera Universitaria Pisana – Pisa (Italy)

ROUND TABLE Anti-histamines in the real-world: pharmacovigilance in the spotlight
Pitolisant, a novel inverse agonist at the H_3 histamine receptor: efficacy and safety
Prof. Valerio Brunetti – Policlinico Gemelli, Università Cattolica del Sacro Cuore – Roma (Italy)

PROGRAMME

23 MAY 2024

09:00 EHRS & HRSE COUNCIL MEETINGS

09:30 Registration

10:45 **OPENING CEREMONY** *Arianna Carolina Rosa (IT) & Katerina Tiligada (EL)*

11:15 Foundation of the HRSE – *Holger Stark (DE) & Detlef Neumann (DE)*

11.30 **PLENARY LECTURE** *Chairperson: Arianna Carolina Rosa (IT)*

Histamine and other biogenic amines in food: from scombroid poisoning to histamine intolerance – *Oriol Comas-Basté (Barcelona, Spain)*

12:30 Welcome buffet

13:15 HONORARY MEMBERSHIP CEREMONY *Chairperson: Katerina Tiligada (EL)*

Orations by Paul Chazot (UK), Agnieszka Fogel (PL), M. Ennis (UK), Detlef Neumann (DE)

14:00 Scientific session I – Neuronal histamine *Chairpersons: Beatrice Passani (IT) & Ling Shan (NL)*

Oral presentations

14:00 The roles of histamine and histamine H_1 receptor in hypocretin neuron development and behavior in zebrafish – *Pertti Panula (Helsinki, Finland)*

14:15 Histaminergic neuronal activity controls food consumption: a chemogenetic study – *Gustavo Provensi (Florence, Italy)*

14:30 Histaminergic neurons modulate vulnerability and resilience to psychological stress – *Beatrice Passani (Florence, Italy)*

14:45 Exploring the interplay of histamine H_3 receptors and mTORC1: Implications for neuropathic pain relief – *Paul Chazot (Durham, UK)*

Flash presentations

15:00 The role of glial cells activation in the analgesic effects of histamine H_3 receptor antagonist, E-98 –*In vivo* and *in vitro* studies – *Katarzyna Popiolek-Barczyk (Krakow, Poland)*

15:10 EHRS & HRSE General Assemblies

17:15 End of first day

SOCIAL PROGRAMME

17:30 Guided City Centre Walk

19:30 Welcome reception

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24 MAY 2024

08:30 Scientific session II – Neuronal histamine *Chairpersons: Pertti Panula (FI) & Gustavo Provensi (IT)*

Oral Presentations

08:30 The roles of histaminergic neural circuits in the precise regulation of CNS disorders and its mechanisms – *Zhong Chen (Hangzhou, China)*

08:45 Postsynaptic histamine H_3 receptors in ventral basal forebrain cholinergic neurons modulate contextual fear memory – *Yanrong Zheng (Hangzhou, China)*

09:00 Whole brain output and input mapping of mouse histaminergic network – *Wenkai Lin (Hangzhou, China)*

09:15 Histamine-4 receptor antagonist inhibits pro-inflammatory microglia and prevents the progression of Parkinson-like pathology: A new therapeutic strategy – *Ling Shan (Amsterdam, The Netherlands)*
09:30 Histamine H_2 receptor deficiency in parvalbumin-positive neurons leads to attention deficit hyperactivity disorder-like phenotypes – *Dadao An (Hangzhou, China)*
Flash Presentations
09:45 Effects of H3R antagonist E169 on autophagy and autism-like behavioral phenotypes in BTBR mouse model of autism – *Nermin Eissa (Abu Dhabi, UAE)*
09:50 The H3R antagonist E159 attenuates neuroinflammation and autistic-like behaviours in BTBR T+ tf/J mouse model of autism – *Nermin Eissa (Abu Dhabi, UAE)*
09:55 An H_2R-dependent medial septum histaminergic circuit mediates feeding behavior – *Lingyu Xu (Hangzhou, China)*
10:00 Histamine receptors: Novel drug targets for schizophrenia– *Weiwei Hu (Hangzhou, China)*

10:05 Coffee break

10:35 GB WEST LECTURE *Chairperson & Introduction of the speaker: Madeleine Ennis (Belfast, UK)*
Mechanisms of mast cell inflammatory mediator secretion: an update – Ulrich Blank *(Paris, France)*

11:35 Scientific session III – Histamine, mast cells & basophils *Chairpersons: Susanne Mommert (DE) & Mitsunobu Mio (JP)*
Oral Presentations
11:35 Oral desensitization to peanut differentially affects the reactivity of human basophils to IgE-dependent stimulation – *Bernhard Gibbs (Canterbury, UK)*
11:50 GPR35 mediates stabilization of mast cells – *Satoshi Tanaka (Kyoto, Japan)*
12:05 Mast cells are differently affected by ST-2309 and ciproxifan, H3R blockers – *Agnieszka Fogel (Lodz, Poland)*
12:20 Scabies mites, what's itching – Does the interplay between parasite-derived pruritogens and host MRGPRX2 explain the intense itching in human scabies? – *Franco Falcone (Giessen, Germany)*

12:35 Scientific session IV – Drug design & molecular targeting *Chairpersons: Rob Leurs (NL) & Paul Chazot (PL)*
Oral Presentations
12:35 Structural basis of agonist recognition at the H_4 receptor – *Rob Leurs (Amsterdam, The Netherlands)*
12:50 Pharmacological characterization of seven human histamine H_3 receptor isoforms – *Meichun Gao (Amsterdam, The Netherlands)*
Flash Presentations
13:05 Distinct G protein and β -arrestin recruitment to seven histamine H_3 receptor isoforms *Meichun Gao (Amsterdam, The Netherlands)*
13:10 Reaching beyond the UV spectrum – A pharmacological profile of a red-shifted H1R photocaged ligand – *Ivana Josimovic (Amsterdam, The Netherlands)*
13:15 Tetrazine-based building blocks for Click chemistry on histamine H_3 receptor ligands – *Martin Moriz Stark (Düsseldorf, Germany)*

13:20 Buffet lunch

14:00 YOUNG INVESTIGATORS AWARDS *YIA Panel: Bernhard Gibbs (UK), Beatrice Passani (IT)*

14:00 Histamine receptor H_4 modulates the expression of Na^+/H^+ (NHE)3 exchanger in the renal proximal tubular cell line HK-2 – *Chiara Gerbino (Turin, Italy)*
14:15 VUF26063: a second-generation photoswitchable ligand to optically control the histamine H_3 receptor – *Ivana Josimovic (Amsterdam, The Netherlands)*
14:30 Multitargeting compounds for neurodegenerative diseases: Dual H3R/ChEs ligands with chelating properties – *Flávia Barrio Lopes (São Paulo, Brazil)*
14:45 Do histaminergic neurons express adenosine 1 receptors? Comparative analysis with reference to hippocampal dentate gyrus granular cells – *Lea Wegmann (Düsseldorf, Germany)*

15:00 Scientific session V in memory of Prof. Pier F. Mannaioni – Histamine in the heart & the kidney *Chairpersons: Anita Sydbom (SE) & Joao Fernandes (BR)*
15:00 Oration in memory of Prof. Pier F. Mannaioni – *Roberto Fantozzi (IT)*
Oral Presentations
15:10 Haloperidol, clozapine and mirtazapine are functional antagonists at histamine receptors in human atrial preparations – *Jonas Schlicht (Halle, Germany)*
Flash Presentations
15:25 Contractile effects of histamine in mice overexpressing H_1-histamine receptors and H_2-histamine receptors in the atrium – *Hoai Pham (Halle, Germany)*
15:30 Temperature sensitive contractile effects of histamine in mice overexpressing H_1-histamine receptors in the atrium – *Peter Grundig (Halle, Germany)*
15:35 Different role of histamine H_1 and H_4 receptors on the expression of aquaporin (AQP)1 and AQP7 in the human renal proximal tubule cell line HK-2 – *Marta Molino (Florence, Italy)*
15:40 Investigation of the cross-talk between histamine and renal CLC chloride channels and transporters in a mouse model of diabetic nephropathy – *Arianna Carolina Rosa (Turin, Italy)*

15:45 Coffee break

16.15 Group picture

16:30 Scientific session VI – Histamine in the eye *Chairpersons: Holger Stark (DE) & & Nermin Eissa (UAE)*
Oral Presentations
16:30 H_4 histamine receptor upregulation and inflammatory responses in an in vitro model of diabetic retinopathy – *Paul Chazot (Durham, UK)*
16:45 Histamine stimulates calcium-dependent transmembrane cation current through H1R-$G_{q/11}$ pathway in human retinal glial Müller cells – *Seva Telezhkin (Newcastle upon Tyne, UK)*
17:00 H3R antagonist-CA inhibitor hybrid compounds in a rabbit model of transient ocular hypertension – *Laura Lucarini (Florence, Italy)*
Flash Presentations
17:15 Evaluation of fibrotic markers in an animal model of ocular hypertension treated with a histamine H3R/NO hybrid compound – *Silvia Sgambellone (Florence, Italy)*
17:20 Photoswithchable compounds in a transient ocular hypertension (OHT) model in new zealand white (NZW) rabbit – *Silvia Marri (Florence, Italy)*
17:25 Study of microRNA involved in ocular ischemia pathway – *Serafina Villano (Florence, Italy)*

17:30 Flash presentation award Panel meeting *Detlef Neumann (DE), Satoshi Tanaka (JP), Katarzyna Popiolek-Barczyk (PL)*

17:30 YIA Panel meeting *Bernhard Gibbs (UK), Beatrice Passani (IT)*

17:45 End of second day

SOCIAL PROGRAMME

20:00 Gala Dinner

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25 MAY 2024

08:45 KEYNOTE LECTURE *Chairperson: Holger Stark (DE)* - 25 years of H_4 receptor research – *Katerina Tiligada (Athens, Greece)*

09:30 Scientific session VII – Histamine in inflammation and immunity *Chairpersons: Agnieszka Fogel (PL) & Laura Lucarini (IT)*

Oral Presentations

09:30 Histamine upregulates the expression of MMP12 in human M2 macrophages – *Susanne Mommert (Hannover, Germany)*

09:45 Assessing the anthelmintic potential of antihistamines H_1 against *Angiostrongylus cantonensis – Joao Paulo Fernandes (São Paulo, Brazil)*

Flash Presentations

10:00 Cyclophosphamide auguments humoral immunity and drug resistance against L-asparaginase – *Mitsunobu Mio (Okayama, Japan)*

10:05 Coffee break

10:30 Round Table · Anti-histamines in the real-world: pharmacovigilance in the spotlight *Moderator: Armando Genazzani (IT)*

10:30 Introduction

10:35 H_1-receptor affinity of psychotropics and risk of weight gain and metabolic disorders – *Virginio Salvi (Milano, Italy)*

10:50 Where has rantidine gone? – *Marco Tuccori (Pisa, Italy)*

11:05 Pitolisant, a novel inverse agonist at the H_3 histamine receptor: efficacy and safety – *Valerio Brunetti (Rome, Italy*)

11:20 Discussion

11:40 Closing remarks – *Rob Leurs (NL) & Arianna Carolina Rosa (IT)*

12:00 End of the congress

INVITED SPEAKERS ABSTRACT

PLENARY LECTURE -

HISTAMINE AND OTHER BIOGENIC AMINES IN FOOD: FROM SCOMBROID POISONING TO HISTAMINE INTOLERANCE

O. Comas-Basté, S. Sánchez-Pérez, M.T. Veciana-Nogués, M.L. Latorre-Moratalla, M. Carmen Vidal-Carou

Histamine is present in a wide range of foods in highly variable concentrations, which are the main exogenous source of this compound. The main route for histamine formation in foods is the decarboxylation of the amino acid histidine through the action of L-histidine decarboxylase, an enzyme of bacterial origin. Therefore, foods that potentially contain high levels of histamine are: a) those microbiologically altered, such as fish and meat, or derived products that may have been preserved or processed in unsuitably hygienic conditions; and b) fermented products, in which the bacteria responsible for the fermentation process may also have aminogenic capacity.

Although histamine has important physiological functions in the body, it can pose a health risk when ingested in high levels. Histamine intoxication is caused by the intake of foods with high levels of histamine. This food intoxication is characterized by occurring in outbreaks and having a short incubation period, with symptoms that are generally of low/moderate severity and remit in a few hours. Although histamine intoxication has been extensively studied in recent decades, unresolved questions remain, concerning, for example, the variable histamine concentrations in the foods triggering outbreaks, or the heterogeneity in the degree and type of adverse effects. On the other hand, histamine intolerance arises from reduced histamine degradation capacity in the intestine due to impaired diamino oxidase (DAO) enzyme activity, leading to its accumulation in plasma and the appearance of adverse effects. The clinical manifestations of histamine intolerance consist of a wide range of nonspecific gastrointestinal and extraintestinal symptoms, which can appear in susceptible individuals after the ingestion of foods containing normal or even low histamine levels. Currently, the main strategy to avoid the symptoms of histamine intolerance is to follow a low-histamine diet, while the supplementation with exogenous DAO enzyme has recently been postulated as a complementary treatment to enhance dietary histamine degradation in the intestines.

Although interest in histamine intolerance has considerably grown in recent years, more scientific evidence is still required to help define, diagnose and clinically manage this condition. In this conference, a glance on histamine intoxication will be performed, as well as the analysis of some uncertainties historically associated to histamine intoxication outbreaks that may be better explained by the existence of interindividual susceptibility to ingested histamine. Moreover, an updated review on histamine intolerance will be delivered, mainly focusing on its etiology and the existing diagnostic and treatment strategies.

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INVITED SPEAKERS ABSTRACT

GBW LECTURE -

MECHANISMS OF MAST CELL INFLAMMATORY MEDIATOR SECRETION: AN UPDATE

Ulrich Blank

Following the milestone finding of J.F. Riley & G.B West recognizing mast cells as the major source of histamine more than 7 decades have past and mast cells are now well recognized actors of inflammatory and immune responses. Besides histamine, they are known to release upon stimulation a plethora of inflammatory mediators prestored either in their cytoplasmic granules or after new synthesis including various lipid compounds as well as cytokines and chemokines. Besides their important task of launching the inflammatory cascade locally in tissues, these mast cell products also contribute to the regulation of the accompanying immune response and the crosstalk with other inflammatory cells. Our group has been studying for many years the signalling events leading to the release of mediators following allergic stimulation through the high affinity IgE receptor (FceRI) focusing on receptor-distal event such as those implicated in the fusion of secretory granules with the plasma membrane. More recently we became also interested in studying the mechanisms regulating the production, secretion and vesicular trafficking of CCL2, also called monocyte chemoattractant protein-1 (MCP-1), a major chemokine produced by mast cells. My talk will provide you with an overview and update on these late signalling and trafficking events governing mast cell mediator release.

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INVITED SPEAKERS ABSTRACT

KEYNOTE LECTURE -

25 YEARS OF HISTAMINE H_4 RECEPTOR RESEARCH

Katerina Tiligada

Histamine has been one of the most studied substances in biomedical sciences since the early 1900s. The recognition of its multifaceted functions in health and disease that are elicited *via* the histamine $H_{1\text{-}4}$ receptors ($H_{1\text{-}4}R$) laid the basis for the development and the clinical exploitation of HR-targeting therapeutics. In 2000, the discovery of the H_4R by exploiting molecular biology approaches revived both the academic and the industrial interest in the field. This novel HR was shown to be primarily expressed in immune cells and to mediate mast cell and eosinophil chemotaxis. Subsequent intense research, largely assisted by the first highly selective H_4R antagonist JNJ7777120 revealed the functional role of the H_4R in immunocompetent cells and in inflammation, and characterized it as the immunomodulatory HR and as a promising emerging target for the treatment of chronic inflammatory diseases. Despite the complex H_4R pharmacology, as exemplified by the β-arrestin bias signalling, and the species- and strain-dependent effects of H_4R ligands in preclinical models of inflammation, a number of H_4R-targeting compounds advanced into clinical testing in patients with atopic dermatitis, pruritus, asthma, rheumatoid arthritis and vestibulopathy. The hitherto failure of H_4R antagonists to enter late clinical trials may hold back the enthusiasm of the big pharma industry to further advance H_4R-targeting compounds to clinical testing. However, the development of selective H_4R ligands remains an ongoing endeavour. Equally, continuing efforts using standard pharmacological approaches and cutting-edge methodologies aim to better understand the (patho)physiological significance of the receptor. These studies would benefit the dissection of H_4R molecular properties, the rational design of new generation H_4R ligands and their value as new therapeutic options for unmet medical needs in inflammation-driven disorders.

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INVITED SPEAKERS ABSTRACT

ROUND TABLE -

H_1-RECEPTOR AFFINITY OF PSYCHOTROPICS AND RISK OF WEIGHT GAIN AND METABOLIC DISORDERS

Virginio Salvi

People with severe mental illness are more frequently affected by obesity, diabetes and dyslipidemias than the general population, eventually resulting in greater cardiovascular disease morbidity and mortality. This is due to several factors such as genetic liability and unhealthy lifestyles; however, the use of psychotropic medication also plays a central role. Specifically, the use of antipsychotics and to a lesser extent antidepressants and mood stabilizers, put the patients at risk for weight gain and metabolic disorders.

Histamine plays a role in inducing satiety through its interaction with histamine H_1-receptors in the hypothalamus. Several antipsychotics are H_1-receptor antagonists, and some studies have highlighted an association between their affinity at histamine H_1-receptor and weight gain. Specifically, antipsychotics such as olanzapine and clozapine, that share potent anti-histaminergic activity, produce a very relevant weight gain in treated patients. These antipsychotics also produce glucose metabolism dysregulation, thus increasing the likelihood of type 2 diabetes. This effect is seen both as a consequence of the weight gain and due to the blockade of histaminergic receptors located on pancreatic beta cells, resulting in a reduced production of insulin.

In more recent years, an association between anti-histaminergic activity and weight gain or metabolic syndrome has been found also for some antidepressant with high H_1-receptor affinity, such as amitriptyline and mirtazapine.

In conclusion, weight gain and metabolic abnormalities observed during treatment with psychotropics is greatly explained by the capacity of these medications to effectively block the histamine H1 receptor, thus promoting food intake and eventually metabolic dysregulation.

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INVITED SPEAKERS ABSTRACT

ROUND TABLE -

WHERE HAS RANITDINE GONE?

Marco Tuccori

Ranitidine has been a widely used medication for decades. It belongs to the class of H2-receptor antagonists and is primarily prescribed to reduce stomach acid production in patients with conditions such as heartburn and stomach ulcers.

In 2019, laboratory tests revealed the presence of N-Nitrosodimethylamine (NDMA), a potentially carcinogenic compound, in ranitidine formulations. NDMA is a probable human carcinogen, and its levels were found to be unacceptable in some batches of ranitidine. Although the exact source of this impurity remains unclear, it is believed that NDMA may form during the degradation of ranitidine, especially when stored at higher temperatures or over time. The impurity levels in some ranitidine formulations were found to increase over time, raising concerns about patient safety.

The discovery prompted regulatory agencies to take action. Major drug regulators, including the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA), suspended the sale of ranitidine in 2020 due to the NDMA contamination. The FDA recommended manufacturers withdraw all prescription and over-the-counter ranitidine drugs from the market immediately.

Despite its suspension in many countries, ranitidine remains freely available in India. It is sold over the counter without requiring a prescription. The low cost and lack of awareness about potential risks contribute to its continued use in the country. A UK-based pharmaceutical company is exploring the possibility of bringing back ranitidine after the first manufacturer of the active ingredient restored its 'certificate of suitability. However, other manufacturers have no plans to reintroduce the product.

In conclusion, the withdrawal of ranitidine underscores the importance of rigorous safety assessments and continuous monitoring of pharmaceutical products quality to protect public health.

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ROUND TABLE -

PITOLISANT, A NOVEL INVERSE AGONIST AT THE H3 HISTAMINE RECEPTOR: EFFICACY AND SAFETY

V. Brunetti

Pitolisant, a selective H3 receptor (H3R) antagonist/inverse agonist, activates histaminergic neurons predominantly located in the cerebral cortex, hypothalamus, hippocampus, and basal ganglia. These neurons play a crucial role in promoting wakefulness and decreasing sleep propensity. Narcolepsy and obstructive sleep apnea syndrome (OSAS) are debilitating sleep disorders characterized by excessive daytime sleepiness (EDS), significantly impacting daily functioning and quality of life.
Narcolepsy, a rare neurological disorder, manifests with EDS and brief episodes of muscle weakness (cataplexy). OSAS, a prevalent disorder, involves intermittent upper airway collapse during sleep, resulting in reduced blood oxygen saturation, sleep fragmentation, and consequent EDS.
Pitolisant has emerged as a promising therapeutic intervention for managing EDS associated with these conditions. Clinical trials consistently demonstrate significant enhancements in both objective and subjective measures of wakefulness in patients with narcolepsy and OSAS.
The safety profile of pitolisant seems favorable, with predominantly mild to moderate and transient adverse events, including headache, insomnia, anxiety, and nausea. Notably, pitolisant lacks the abuse potential and cardiovascular risks often linked with conventional wake-promoting agents.
Moreover, pitolisant demonstrates minimal potential for drug-drug interactions, facilitating coadministration with other medications commonly used in narcolepsy and OSAS.
In conclusion, pitolisant presents a promising therapeutic option for addressing sleepiness in narcolepsy and OSAS, offering both efficacy and safety advantages. Further long-term investigations are warranted to elucidate its sustained efficacy and safety profile, particularly in real-world clinical scenarios.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION I – NEURONAL HISTAMINE

THE ROLES OF HISTAMINE AND HISTAMINE H1 RECEPTOR IN HYPOCRETIN NEURON DEVELOPMENT AND BEHAVIOR IN ZEBRAFISH

P. Panula, P. Chalas, Y. Yao, Y.-C. Chen, M. Sundvik

Histamine and hypocretin (hcrt) systems are closely linked hypothalamic regulators of e.g. alertness and sleep in vertebrates including humans. The number of neurons expressing the histamine-synthesizing enzyme histidine decarboxylase (hdc) has been reported to be abnormally high in narcoleptic patients, whereas the number of hcrt cells is strongly reduced.
Using gene-modified zebrafish generated with the CRISPR-Cas method lacking either H1 receptor (*hrh1*) or *hdc,* we analyzed the number of hypothalamic hypocretin neurons using immunohistochemistry for hypocretin and *in situ* hybridization for *hcrt* mRNA. Quantitative behavioral methods were used to assess sleep-like behavior and motor responses in 6-7 days post fertilization (dpf) zebrafish larvae.
Both *hdc*$^{-/-}$ and *hrh1*$^{-/-}$ larvae displayed largely normal (similar to wild-type littermates) sleep and motor behavior. Both *hdc*$^{-/-}$ and *hrh1*$^{-/-}$ larvae had abnormally few *hcrt* neurons in the brain. In *hrh1*$^{-/-}$ zebrafish, the number of *hcrt* neurons was abnormally low during the early larval stage and at 20 dpf, but did not differ from wild-type fish during adulthood, suggesting delayed development.
The results suggest that the histamine system and hrh1 are not needed for normal sleep-like resting state and motor behavior in zebrafish. Histamine and hrh1 are required for normal early development of *hcrt* neurons, in contrast with previously published results on other models of zebrafish lacking hrh1 or hdc, but in agreement with our previous results using treatment of larvae with hrh1 inverse agonist pyrilamine or alpha-methylfluorohistidine, a suicide inhibitor of hdc.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION I – NEURONAL HISTAMINE

HISTAMINERGIC NEURONAL ACTIVITY CONTROLS FOOD CONSUMPTION: A CHEMOGENETIC STUDY

A. Costa, V. Sordi, M.B. Passani, G. Provensi

Food consumption is regulated by two systems: the *homeostatic*, driving the motivation to eat following depletion of energy stores, and the *hedonic,* modulating the desire to eat, particularly palatable foods, even in periods of energy abundance. Previous reports revealed that neuronal histamine (HA) plays a role on feeding behavior, however, most studies were based on genetic or pharmacological manipulations which present technical limitations. We aimed to investigate the impact of modulating endogenous HA neurotransmission on food consumption using a chemogenetic approach. Mice expressing a Cre recombinase under histidine decarboxylase promoter control (*Hdc*-Cre) received bilateral injections of viral vectors carrying Cre-dependent excitatory (hM3Dq) or inhibitory (hM4Di) designer receptors exclusively activated by designer drugs (DREADDs) into the turberomamillary nucleus. Four weeks after injections, mice received systemic administration of DREADDs ligand Clozapine-N-Oxide (CNO, 2 mg/kg, i.p.). Normal or palatable (60% fat) chow intake was measured in the fasting-refeeding paradigm. Opposite effects emerged following acute HA system modulation: silencing of HA neurons elicited food intake, whereas their activation suppressed food intake. Repeated CNO administration (2 mg/kg, once daily, 10 days) to mice expressing excitatory DREADDs not only reduced the cumulative amount of food consumed but also determined a significant reduction of mice' body weight. Finally, chemogenetic stimulation of HA neurons did not elicited aversion in the conditional place preference task, suggesting that the decrease in food intake is not caused by nausea or malaise. In conclusion, our results confirm and expand previous reports regarding the role of neuronal HA in regulating feeding behaviour and pave the way for future studies to deconstruct specific histaminergic neural pathways involved, as well as regulatory mechanisms underlying feeding control.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION I – NEURONAL HISTAMINE

HISTAMINERGIC NEURONS MODULATE VULNERABILITY AND RESILIENCE TO PSYCHOLOGICAL STRESS

A. Costa, V. Sordi, G. Provensi, M.B. Passani

Exposure to stress represents a major condition conferring risk for different psychiatric disorders, characterised by dysfunctions of cognitive, emotional, and social domains. However, not everyone who experiences an adverse event succumbs to negative outcomes and enters a pathological state, as some individuals are highly vulnerable to the pathological consequences of stress exposure, whereas others appear to be resilient. In this regard, knowledge of TMN histamine (TMN^{HA}) neurons role in stress vulnerability and resilience is missing. To understand the involvement of TMN^{HA} neurons in the development of stress-induced impairments, mice underwent an ethologically valid stress protocol, the chronic social defeat stress (CSDS) and tested for cognitive impairments. To question the effect of activating/inhibiting TMN^{HA} neurons, chemogenetic, pharmacological and genetic approaches were used: 1 Cre-dependent excitatory (hM3Dq) or inhibitory (hM4Di) viral constructs were injected in the TMN of Hdc-Cre mice and the activator clozapine-NO (CNO) injected i.p. during the CSDS; 2) the H_3R antagonist pitolisant (10mg/kg i.p.) or the agonist VUF16839 (5 mg/kg i.p.) were injected during the CSDS; 3) *Hdc*-ko and WT mice were subjected to the CSDS. Behavioural consequences of CSDS were evaluated with non-rewarded, spontaneous social and learning tasks and compared to those of non-stressed mice. mRNA levels of HA producing/metabolizing enzymes and HA receptors were analysed in the TMN, hippocampus and frontal cortex of stressed *versus* control mice. We found alterations of HA receptors and metabolic enzymes mRNA transcripts in the brain of stressed mice. Crucially, activation of TMN^{HA} neurons promoted resilience to CSDS-induced social and cognitive impairments, whereas chemogenetic, genetic or pharmacological silencing of TMN^{HA} neurons induced vulnerability. Our results reveal that interventions targeting the HA system promotes coping and long-term resilience by normalizing stress-affected behaviours

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION I – NEURONAL HISTAMINE

EXPLORING THE INTERPLAY OF HISTAMINE H_3 RECEPTORS AND mTORC1: IMPLICATIONS FOR NEUROPATHIC PAIN RELIEF

I. Obara, I. Alrashdi, P. Chazot

Histamine H_3 receptors (H_3Rs), which are expressed in the central nervous system (CNS), seem to play a significant role in the regulation of chronic pain processes. However, the exact mechanisms explaining the involvement of H_3Rs in the regulation of neuropathic pain that develops after nerve injury are not well understood. One of the proposed mechanisms involves the inhibition of the Akt/GSK-3β pathway, as well as the subsequent inhibition of the mammalian target of rapamycin complex 1 (mTORC1) pathway, resulting from the H_3Rs antagonism and leading to neuroprotective effects. Thus, the present study focused on the possible interactions between H_3R and mTORC1 and its downstream effectors that may have implications in the regulation of neuropathic pain symptoms. Adult male C57BL/6J mice (n=6/group) were subjected to peripheral neuropathy induced by chronic constriction injury (CCI) of the sciatic nerve and were treated with a CNS-sparing H_3R antagonist/reversible inverse agonist, PF-0868087 (10 mg/kg once every 24 h for 4 days, i.p.; Novartis). Control animals were not subjected to CCI (shams) and/or were treated with 0.1 M citrate buffer (vehicle). Western blot analyses, performed on the L4-L6 spinal cord and sciatic nerve samples, showed that PF-0868087 reduced mTORC1 activity in neuropathic mice, as observed by the inhibition of ribosomal S6 proteins, p70 ribosomal protein S6 kinase and eukaryotic initiation factor 4E-binding protein. This data strongly aligns with our earlier behavioural studies showing antinociceptive efficacy of PF-0868087 in neuropathic mice and emphasises the significant role of the mTORC1 pathway in the antinociceptive effect of H_3R antagonists, suggesting a histamine-independent mechanism. The present findings, therefore, shed new light on H_3R and its signalling pathways in the context of neuropathic pain and suggest that H_3R is a potential target in therapy for this type of chronic pain, where mTORC1 signalling may play a critical role.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION I – NEURONAL HISTAMINE

THE ROLE OF GLIAL CELLS ACTIVATION IN THE ANALGESIC EFFECTS OF HISTAMINE H_3 RECEPTOR ANTAGONIST, E-98 – *IN VIVO* AND *IN VITRO* STUDIES.

Katarzyna Popiolek-Barczyk[*], *Maciej Degutis, Dorota Łażewska, Justyna Barut, Magdalena Białoń, Żaneta Broniowska, Gniewomir Latacz, Katarzyna Szczepańska, Tadeusz Karcz, Katarzyna Kieć-Kononowicz, Katarzyna Starowicz*

Neuropathic pain is a pathological disorder caused by lesions or diseases of the somatosensory nervous system, where profound central inflammation is observed, involving the CNS-resident non-neuronal cells, mainly microglia and astrocytes. A growing body of evidence indicates the histaminergic system is a therapeutic target for pain management. Our study aimed to determine the analgesic effects of histamine H_3 receptor (H_3R) antagonist in neuropathic mice and its influence on glia activation, which might underpin its potential efficacy. We used chronic constriction injury (CCI) to model neuropathic pain. We investigated the effects of novel H_3R antagonist, E-98 1-(7-(4-chlorophenoxy)heptyl)-3-methylpiperidine)) after single injection (1, 5, 10, 20 mg/kg) on mechanical (von Frey) and thermal (cold plate) stimuli in CCI-exposed (day 14^{th}) mice, along with the possible participation of spinal H_1R, H_2R, and H_4R. Moreover, the effect of chronic E-98 (10 mg/kg) treatment (twice daily, 7 days) was investigated, and its influence on glia activation in the lumbar spinal cord (Western blot). The anti-inflammatory properties of E-98 (10 μM) were screened (ELISA) in microglia and astrocytes primary cell cultures. We assessed the presence of H_3R in the spinal cord of neuropathic mice and in the primary cultures of glial cells. E-98 attenuated nociceptive responses and this effect was reversed by pretreatment with pyrilamine, an H_1R antagonist, and ranitidine, an H_2R antagonist, but not JNJ7777120, an H_4R antagonist. Chronic treatment also produced potent analgesia correlated with reduced microglia, but not astroglia, activation. *In vitro* studies showed a decreased IL-6 level in both glial cell cultures. We observed co-localization of H_3R with neurons, as well as microglia and astrocytes, in the spinal cord and cell cultures. Our studies explore the mechanism of analgesic action of H_3R antagonists and might help identify innovative therapeutic interventions for neuropathic pain.

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SCIENTIFIC SESSION II – NEURONAL HISTAMINE

THE ROLES OF HISTAMINERGIC NEURAL CIRCUITS IN THE PRECISE REGULATION OF CNS DISORDERS AND ITS MECHANISMS

Zhong Chen

Histamine is an important neurotransmitter in the brain. Recently, my team has employed the fluorescence micro-optical sectioning tomography (fMOST) technique to construct the whole-brain mapping of histaminergic projections in mice (PNAS, 2023a), based on which we further analyze the differential roles of different histaminergic neural projections in feeding (Curr Biol, 2022), fear memory (Cell Rep, 2023) and various behaviors. Meanwhile, by using Cre-Loxp technology to selectively deplete histamine receptors in different brain regions and types of cells, we revealed the cell-type-specific characteristics of histamine receptors in schizophrenia (Nat Commun, 2021; PNAS, 2023b) and cerebral ischemia (J Exp Med, 2021). In addition, proteomic techniques are employed to identify downstream signaling pathways of histamine receptors and found the critical role of autophagy in the intracellular regulation by histamine H3 receptor (Nat Commun, 2014; Autophagy, 2014; 2017a; 2017b; JCB, 2019). The above studies systematically elucidate the roles of histaminergic neural projections in different CNS disorders and reveal the cell-type-specific functions of histamine receptors, which may shed light on future precise interventions for CNS disorders.

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SCIENTIFIC SESSION II – NEURONAL HISTAMINE

POSTSYNAPTIC HISTAMINE H3 RECEPTORS IN VENTRAL BASAL FOREBRAIN CHOLINERGIC NEURONS MODULATE CONTEXTUAL FEAR MEMORY

Yanrong Zheng, Zhong Chen

Overly strong fear memories can cause pathological conditions. Histamine H3 receptor (H_3R) has been viewed as an optimal drug target for CNS disorders, but its role in fear memory remains elusive. We find that a selective deficit of H_3R in cholinergic neurons, but not in glutamatergic neurons, enhances freezing level during contextual fear memory retrieval without affecting cued memory. Consistently, genetically knocking down H_3R or chemogenetically activating cholinergic neurons in the ventral basal forebrain (vBF) mimics this enhanced fear memory, whereas the freezing augmentation is rescued by re-expressing H_3R or chemogenetic inhibition of vBF cholinergic neurons. Spatiotemporal regulation of H_3R by a light-sensitive rhodopsin-H_3R fusion protein suggests that postsynaptic H_3Rs in vBF cholinergic neurons, but not presynaptic H_3Rs of cholinergic projections in the dorsal hippocampus, are responsible for modulating contextual fear memory. Therefore, precise modulation of H_3R in a cell-type- and subcellular-location-specific manner should be explored for pathological fear memory.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION II – NEURONAL HISTAMINE

WHOLE BRAIN OUTPUT AND INPUT MAPPING OF MOUSE HISTAMINERGIC NETWORK

*W. Lin, Y. Wang, Z. Chen**

Histamine is a conserved neuromodulator in mammalian brains and critically involved in many physiological functions. Understanding the precise structure of histaminergic output and input network is the cornerstone in elucidating its function.

Herein, using novel HDC-CreERT2 mice and genetic labeling strategies, we reconstructed whole brain 3D structure of histaminergic neurons and their output and input circuits at $0.32\times0.32\times2$ μm^3 pixel resolution with a cutting-edge fluorescence micro-optical sectioning tomography system (fMOST).

Based on the data of whole-brain histaminergic output map, we quantified the fluorescence-labeled fiber density of all brain areas and found that histaminergic fiber density varied significantly among brain regions. The density of histaminergic fiber was positively correlated with the amount of histamine release induced by optogenetic stimulation or physiological aversive stimulation. Moreover, we reconstructed fine morphological structure of 60 histaminergic neurons via sparse labeling, and uncovered the largely heterogeneous projection pattern of individual histaminergic neuron. On the other hand, wo also analyzed whole-brain input map of histaminergic neurons, and found that most upstream brain regions located in the hypothalamus. Besides, some nuclei of thalamus, striatum, midbrain, hippocampus, pallidum, and cortical subplate also provide dense inputs to histaminergic neurons. Interestingly, there are specific layer distribution of upstream cortical neurons, and their co-projection pattern was completely analyzed. Then, the excitatory and inhibitory components of neurons in the main upstream brain regions were determined using in situ hybridization staining. Lastly, we selected paraventricular nucleus of the thalamus (PVT), and lateral septal nucleus (LS) as representative of excitatory and inhibitory brain regions, which show differential electrophysiological functions to histaminergic neurons and dynamic characteristics of neuronal activity in sleep-wake rhythm.

Collectively, this study reveals an unprecedented whole-brain quantitative analysis of histaminergic projections and upstream inputs at the mesoscopic and microscopic circuit level, providing a foundation for shedding light on histamine's role not only in physiologic function but also CNS disorders.

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HISTAMINE-4 RECEPTOR ANTAGONIST INHIBITS PRO-INFLAMMATORY MICROGLIA AND PREVENTS THE PROGRESSION OF PARKINSON-LIKE PATHOLOGY: A NEW THERAPEUTIC STRATEGY

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Activation of microglia is presumed to play a key role in the pathogenesis of Parkinson's disease (PD). The activity of microglia is regulated by the histamine-4 receptor (H_4R), thus providing a novel target that may prevent the progression of PD. However, this putative mechanism has so far not been validated. In our previous study, we found that mRNA expression of H_4R was upregulated in the basal ganglia of PD patients. In the current study, upregulation of H_4R-mRNA, stands out by robustness and consistency in an independent genome-wide RNA sequencing study of basal ganglia of PD post-mortem brains. We validated this possible mechanism using the rotenone-induced PD rat model, in which mRNA expression levels of H_4R-, and microglial markers were significantly increased in the substantia nigra pars compacta (SNpc). Inhibition of H_4R in rotenone-induced PD rat model by infusion of the specific H_4R antagonist JNJ7777120 (JNJ) into the lateral ventricle resulted in blockade of microglial activation. In addition, pharmacological targeting of H_4R in rotenone-lesioned rats resulted in reduced apomorphine-induced rotational behaviour and prevention of dopaminergic neuron degeneration and associated decreases in striatal dopamine levels. These changes were accompanied by a reduction of Lewy body-like neuropathology in both SNpc and striatum. Moreover, other neurotransmitters including γ-aminobutyric acid, glutamine, serotonin were relatively stable under the JNJ treatment. Our results provide first proof of the efficacy of an H_4R antagonist in a PD rat model, and proposes the H_4R as a promising target to clinically tackle microglial activation and thereby the progression of PD.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION II – NEURONAL HISTAMINE

HISTAMINE H2 RECEPTOR DEFICIENCY IN PARVALBUMIN-POSITIVE NEURONS LEADS TO ATTENTION DEFICIT HYPERACTIVITY DISORDER-LIKE PHENOTYPES

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Attention deficit hyperactivity disorder (ADHD) is a behavioral disorder with a prevalence of 4%, characterized by symptoms of inattention, hyperactivity, and impulsivity. Currently, the neurophysiological mechanism underlying ADHD remains unclear. Moreover, first-line drug treatments for ADHD are associated with serious side effects due to their nonspecific effects and the presence of inverted-U-shaped dose-effect curves. Here, we discovered that deficiency of histamine H2R in parvalbumin-positive (PV+) neurons induce ADHD-like phenotypes, including hyperactivity, impulsivity, and inattention. Selective knockdown of H2R in PV+ neurons in the substantia nigra pars recticulata (SNr), but not in medial prefrontal cortex, resulted in ADHD-like behavioral disorders by attenuating PV+ neuronal activity. However, H2R overexpression in SNr did not affect locomotion or attention. We observed decreased H2R expression in SNr PV+ neurons in dopamine transporter (DAT)-deficient mice, an animal model for ADHD and their behavioral phenotypes were relieved by treatment with an H2R agonist. Our findings also reveal that H2R deficiency in PV+ neurons induces dysfunction of neuronal efferents from SNr PV+ neurons to the substantia nigra pars compacta (SNc) dopaminergic neurons, contributing only to impaired attention. In addition, efferents from SNr PV+ neurons to intermediate layer of superior colliculus (mSC) contribute to both hyperactivity and inattention. Collectively, these results demonstrate that H2R deficiency in PV+ neurons plays a critical role in the pathogenesis of ADHD by dampening the activity of SNr PV+ neurons and involves different efferents. These findings pinpoint the critical cell type, brain region, and neural circuits through which H2R participates in the pathogenesis of ADHD, which enhances our understanding of the molecular basis of ADHD and identifies H2R in SNr PV+ neurons as a precise therapeutic target in ADHD.

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EFFECTS OF H3R ANTAGONIST E169 ON AUTOPHAGY AND AUTISM-LIKE BEHAVIORAL PHENOTYPES IN BTBR MOUSE MODEL OF AUTISM

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Accumulation of evidence suggests that autophagy is involved in the pathogenesis of autism spectrum disorder (ASD). Akt-mTOR pathway is an important signaling cascade associated with long-term plasticity and thus may contribute to memory and cognitive dysfunction, suggesting its dysregulation in ASD. In addition, mTOR has been widely implicated in autophagy activation, which is one of the important autophagy induction regulators. It is suggested that H3R blockade may be neuroprotective in ASD, as autophagy is reported to be impaired in ASD. Hence, the effects of acute treatment with the novel H3R antagonist E169 (2.5, 5, and 10 mg/kg, i.p.) on short-term memory (STM), long-term memory (LTM), and anxiety levels in male BTBR mice were evaluated using Novel object recognition test (NORT) and open field locomotor test (OFLT) tests. E169 (2.5 mg/kg, i.p) provided significant memory-improving effects on BTBR STM and LTM impairments in NORT.

E169 (2.5 mg/kg, i.p)-provided effects were comparable to those observed with the reference mTOR inhibitor rapamycin and was abrogated with the CNS-penetrant H3R agonist (R)-α-methylhistamine. Our results demonstrated that E169 ameliorated BTBR memory deficits by antagonism of H3Rs and modulation of the disturbed expression of Akt, mTOR, and LC-3 proteins, signifying that E169 regulated the Akt-mTOR signaling pathway in the cerebellum of tested mice. The results revealed that E169 (2.5 mg/kg, i.p.) restored the abnormal anxiety and hyperactivity observed in OFLT.

These findings indicate that H3R antagonists like E169, which possess favorable in silico physicochemical characteristics and maintain crucial interactions in docking studies at H3R, could play a role in simultaneously regulating disrupted brain neurotransmitters and the dysregulated Akt-mTOR signaling pathway associated with autophagy in neurological diseases. This may add a new therapeutic management strategy for memory and cognitive impairment-associated disorders including ASD.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION II – NEURONAL HISTAMINE

THE H3R ANTAGONIST E159 ATTENUATES NEUROINFLAMMATION AND AUTISTIC-LIKE BEHAVIOURS IN BTBR T+ tf/J MOUSE MODEL OF AUTISM

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Large body of evidence suggests the involvement of cognitive impairment, increased levels of inflammation and oxidative stress in the pathogenesis in autism spectrum disorder (ASD). ASD commonly coexists with psychiatric conditions like anxiety and cognitive challenges, and individuals with ASD exhibit significant levels of inflammation and immune system dysregulation. Studies have identified elevated levels of pro-inflammatory markers such as IL-1β, IL-6, IL-2 and TNF-α, particularly in young children with ASD. The current therapeutic options for ASD show limited effectiveness, signifying the importance of exploring novel and efficient drugs to address the core symptoms. The role of histamine H3 receptors (H3Rs) in memory and the prospective role of H3R antagonists in pharmacological control of neurodegenerative disorders, e.g., ASD is well-accepted. Hence, the effects of chronic systemic administration of H3R antagonist E159 and on autistic-like repetitive behaviors, social deficits, memory and anxiety parameters as well as neuroinflammation in Black and Tan BRachyury (BTBR) mice were evaluated using Y maze, Barnes maze, self-grooming, open field and three chamber social test. E159 (2.5, 5 and 10 mg/kg, i.p.) dose-dependently ameliorated repetitive and compulsive behaviors by reducing the increased time spent in self-grooming and improved reduced spontaneous alternation in BTBR mice. Moreover, treatment with E159 attenuated disturbed anxiety levels and social deficits in tested male BTBR mice. Furthermore, E159 attenuated oxidative stress by significantly increasing GSH, CAT, and SOD, and decreasing the increased levels of MDA in the cerebellum as well as the hippocampus. In addition, E159 decreased the elevated levels of proinflammatory cytokines (tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and IL-6). The observed results show that H3R antagonists like E159 may represent a promising novel pharmacological strategy for the future treatment of ASD.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION II – NEURONAL HISTAMINE

AN H2R-DEPENDENT MEDIAL SEPTUM HISTAMINERGIC CIRCUIT MEDIATES FEEDING BEHAVIOR

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Novel targets for treating feeding-related diseases are of great importance, and histamine has long been considered an anorexigenic agent. However, understanding its functions in feeding in a circuit-specific way is still limited. Here, we report a medial septum (MS)-projecting histaminergic circuit mediating feeding behavior. This MS-projecting histaminergic circuit is functionally inhibited during food consumption, and bidirectionally modulates feeding behavior via downstream H2, but not H1, receptors on MS glutamatergic neurons. Further, we observed a pathological decrease of histamine 2 receptors (H2Rs) expression in MS glutamatergic neurons in diet-induced obesity (DIO) mice. Genetically, down-regulation of H2Rs expression in MS glutamatergic neurons accelerates body-weight gain. Importantly, chronic activation of H2Rs in MS glutamatergic neurons (with its clinical agonist amthamine) significantly slowed down the body-weight gain in DIO mice, providing a possible clinical utility to treat obesity. Together, our results demonstrate that this MS-projecting histaminergic circuit is critically involved in feeding, and H2Rs in MS glutamatergic neurons is a promising target for treating body-weight problems.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION II – NEURONAL HISTAMINE

HISTAMINE RECEPTORS: NOVEL DRUG TARGETS FOR SCHIZOPHRENIA

Qianyi Ma, Li Cheng, Lei Jiang, Zhong Chen, Weiwei HU

Schizophrenia is a serious mental disorder, and existing antipsychotic drugs show limited efficacy and cause unwanted side effects, that could be due to non-precise targeting. Histamine in the brain is a neurotransmitter involved in many pathophysiological processes including waking-sleeping, food intake, learning, and memory, and it primarily functions through the histamine H_1 receptor (H_1R). Although H_2 receptors (H_2Rs) are also widespread in the brain, their functions are not well understood. By Cre-loxp system, chemogenetic approach, electrophysiology and human brain samples, we found that H_1R deficiency in basal forebrain cholinergic neurons is critical for schizophrenia-like negative symptoms, however, the H_1R deficiency in dopaminergic neurons and glutamatergic neurons did not induce such behavioral disorder. Interestingly, the H_2R deficiency in the prefrontal cortex glutamatergic neurons is critical for behaviors resembling both negative and both symptoms in schizophrenia through a downregulation of neuronal firing by modulating HCN currents. H_2R agonists can be used to treat schizophrenia-like phenotypes in an MK-801-induced mouse model of schizophrenia. These studies provide histamine receptors as novel drug targets for schizophrenia and also established the histamine receptor hypothesis for the pathogenesis of schizophrenia and improve the understanding of the functional role of H_2R in the brain, especially in glutamatergic neurons.

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SCIENTIFIC SESSION III – HISTAMINE, MAST CELLS & BASOPHILS

ORAL DESENSITIZATION TO PEANUT DIFFERENTIALLY AFFECTS THE REACTIVITY OF HUMAN BASOPHILS TO IgE-DEPENDENT STIMULATION

B.F. Gibbs, M.H. Shamji, N. Patel, P.J. Turner

Basophils are likely to play a key role in determining the severity of allergic reactions, including anaphylactic shock, through the rapid release of histamine and lipid mediators upon allergen-mediated activation. Allergen immunotherapy is very successful in preventing basophil degranulation for venom and drug-induced reactions, but less straightforward for peanut allergy and associated with significant adverse events including anaphylaxis. To overcome this, we used an immunotherapy protocol with initial updosing using boiled rather than roasted peanuts to achieve desensitization. Our aims were to observe whether this approach reduces peanut allergen-induced histamine release from basophils obtained from seven children with severe peanut allergy (NHS HRA approval reference 15/LO/0287). Basophils were purified from peripheral blood using negative immunomagnetic selection before incubation in the presence or absence of peanut allergen, anti-IgE or fMLP. Histamine release was assessed by spectrofluorometric autoanalysis. In five donors, immunotherapy resulted in a striking reduction in basophil histamine release to peanut allergen after 1 year of immunotherapy (from 41.7±8% to 13.5±6.3%). Anti-IgE-dependent basophil reactivity was also moderately reduced in these five donors following allergen immunotherapy (from 43.1±10% to 31.6±11.8%), but not fMLP. In contrast, immunotherapy failed to reduce either peanut allergen- or anti-IgE-induced histamine release in two donors which also displayed the highest histamine release (>65%) to peanut before immunotherapy. These investigations highlight that a population of high basophil responders are resistant to initial allergen immunotherapy, underlining the need for new therapeutic strategies which target signalling pathways responsible for hyperreactive basophils.

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GPR35 MEDIATES STABILIZATION OF MAST CELLS

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Background: Mast cell stabilizers including sodium cromoglycate have been used as useful anti-allergic drugs, although their molecular targets remain to be determined. Recent studies demonstrated that they could act on GPR35, an orphan G protein-coupled receptor, as the agonists. We investigated whether GPR35 was expressed in mast cells and clarified how GPR35 suppresses degranulation of mast cells.
Methods: We used rat purified peritoneal mast cells, and murine IL-3-dependent bone marrow-derived cultured mast cells (BMMCs) as mast cell models. Because GPR35 was absent in a rat basophilic mast cell line, RBL-2H3, we prepared a cloned cell line constitutively expressing rat GPR35 cDNA (RBL-2H3/HA-rat GPR35). IgE-dependent immediate responses were assessed by the passive cutaneous anaphylaxis (PCA) model.
Results: Previously reported GPR35 agonists and newly developed agonists suppressed degranulation of rat peritoneal mast cells upon IgE-mediated antigen stimulation. Decreased levels of degranulation were found in RBL-2H3/HA-rat GPR35 when it was stimulated by IgE/Ag stimulation whereas no significant changes were observed when they were activated by thapsigargin. A GPR35 agonist, zaprinast, induced transient activation of RhoA and a transient decrease in the amount of filamentous actin. No significant changes were observed in PCA responses in the GPR35-deficient mice whereas therapeutic effects of the GPR35 agonists, such as disodium cromoglycate, were abrogated in the GPR35-deficient mice.
Conclusions: GPR35 expressed in rat and mouse mast cells mediates suppression of degranulation induced by IgE-mediated antigen stimulation. GPR35-mediated RhoA activation may be involved in the suppression of mast cell degranulation through cortical actin reorganization.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION III – HISTAMINE, MAST CELLS & BASOPHILS

MAST CELLS ARE DIFFERENTLY AFFECTED BY ST-2309 AND CIPROXIFAN, H3R BLOCKERS

A Walczak-Drzewiecka, WA Fogel, H Stark, J Dastych

The current approach in treating neurodegenerative diseases involves the use of multitarget drugs that contain various groups capable of counteracting different aspects of the disease's pathology. Multitargeting drug candidates being thoroughly explored are these containing the histamine H3 receptor antagonist/inverse agonist, as the core of their chemical structure. An example is peptide-like ST-2309 (3,6-bis(4-(3-(piperidin-1-yl)propoxy)benzyl)piperazine-2,5-dione). All neurodegenerative diseases are characterized by neuroinflammation. There is a shred of growing evidence suggesting that the activation of microglia cells and astrocytes as well as the permeability of the blood-brain barrier are triggered by the activated mast cells (MC) and their released meditators.

The effect of ST-2309 on mast cells and its comparison with that of ciproxifan, an H3R antagonist, was investigated.

The human MC line LAD2, was a gift from Dr. A. S. Kirshenbaum (NIH, Bethesda, MD). Cells were incubated at 37 °C in 5% CO2 in a culture medium, at density of 500,000 cells/mL, in the presence of ST-2309 or Ciproxifan for 20 h. Aliquots of isolated total RNA were reverse-transcribed using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific) in the presence of oligo-dT18 primers. The levels of specific gene expression were measured by real-time PCR amplification. The relative gene expression was calculated using ΔΔC T method with the geometric mean of selected reference genes.

ST-2309 treated LAD2 mast cells showed a significant increase in gene expression of cytokines IL9, IL10 and TNF-α at 20 μM, in contrast to ciproxifan treated cells, which showed a significant reduction in gene expression of IL1A and IL16 at 0.1 μM, and additionally of IL10, TGFB and CMA1 at 10 μM. It is proposed that activation of proinflammatory cytokine genes expression is related to other than H3R blocking moiety of the multitarget compound.

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SCABIES MITES, WHAT'S ITCHING - DOES THE INTERPLAY BETWEEN PARASITE-DERIVED PRURITOGENS AND HOST MRGPRX2 EXPLAIN THE INTENSE ITCHING IN HUMAN SCABIES?

Franco H. Falcone, Prema S. Prakash, Michael H. W. Weber, Bernardo P. Moreira

The most salient feature of scabies, caused by *Sarcoptes scabiei,* is its intense itch, which is poorly responsive to anti-histamine treatment. Use of acaricides leads to week-long post-treatment itch exacerbation. We demonstrate how a *Sarcoptes scabiei*-derived protein and its derived peptides can activate human Mas-related G-protein coupled receptor member X2 (MRGPRX2).

Sarcoptes scabiei–derived Ss-14 was recombinantly expressed in Sf21 insect cells in serum-free medium, purified by affinity chromatography and tested for activation of a fluorescent RBL reporter cell line stably transfected with MRGPRX2.

Ss-14 and derived Lysine/Glutamic acid-rich peptides induced fast degranulation of MRGPRX2 reporter cells in nanomolar concentration. DeepLearning-based *in silico* structure prediction using the AlphaFold2 multimer approach was used to model tight binding of Ss-14-derived peptides to the ligand binding pocket.

This is the first report of a parasite-derived molecule activating MRGPRX2 in the nanomolar range, highlighting MRGPRX2 as a promising novel target for prevention of post-treatment itch exacerbation in human scabies.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION IV – DRUG DESIGN & MOLECULAR TARGETING

STRUCTURAL BASIS OF AGONIST RECOGNITION AT THE H_4 RECEPTOR

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The histamine H_4 receptor (H_4R) plays key role in immune cell function and is a highly valued target for treating allergic and inflammatory diseases. However, structural information of H_4R remains elusive. Here, we report four cryo-EM structures of H_4R/G_i complexes, with either histamine or small molecule agonists clobenpropit, VUF6884 and clozapine bound. Combined with mutagenesis, ligand binding and functional assays, the structural data reveal a distinct ligand binding mode where $D94^{3.32}$ and a π-π network determine the orientation of the positively charged group of ligands, while $E182^{5.46}$, located at the opposite end of the ligand binding pocket, plays a key role in regulating receptor activity. The structural insight into H_4R ligand binding allows us to identify mutants at $E182^{5.46}$ for which the agonist clobenpropit acts as an inverse agonist and to correctly predict inverse agonism of a closely related analogue with nanomolar potency. Together with the findings regarding receptor activation and G_i engagement, we established a framework for understanding H_4R signaling and provide a rational basis for designing novel antihistamines targeting H_4R.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION IV – DRUG DESIGN & MOLECULAR TARGETING

PHARMACOLOGICAL CHARACTERIZATION OF SEVEN HUMAN HISTAMINE H_3 RECEPTOR ISOFORMS

Meichun Gao, Mabel E. Dekker, Rob Leurs and Henry F. Vischer

The histamine H_3 receptor (H_3R) regulates as a presynaptic G protein-coupled receptor the release of histamine and other neurotransmitters in the brain, and is consequently a potential therapeutic target for neuronal disorders. The human H_3R encodes for seven splice variants that vary in the length of intracellular loop 3 and/or the C-terminal tail but are all able to induce heterotrimeric G_i protein signaling. The last two decades, H_3R drug discovery and lead optimization has been exclusively focused on the 445 amino acids-long reference isoform H_3R- 445.

In this study, we pharmacologically characterized for the first time all seven H_3R isoforms by determining their binding affinities for reference histamine H_3 receptor agonists and inverse agonists. The H_3R-453, H_3R-415, and H_3R-413 isoforms display similar binding affinities for all ligands as the H_3R-445. However, increased agonist binding affinities were observed for the three shorter isoforms H_3R-329, H_3R-365, and H_3R-373, whereas inverse agonists such as the approved anti-narcolepsy drug pitolisant (Wakix®) displayed significantly decreased binding affinities for the latter two isoforms. This opposite change in binding affinity of agonist versus inverse agonists on H_3R-365 and H_3R-373 is associated with their higher constitutive activity in a cAMP biosensor assay as compared to the other 5 isoforms. The observed differences in pharmacology between longer and shorter H_3R isoforms should be considered in future drug discovery programs.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION IV – DRUG DESIGN & MOLECULAR TARGETING

DISTINCT G PROTEIN AND β -ARRESTIN RECRUITMENT TO SEVEN HISTAMINE H_3 RECEPTOR ISOFORMS

Meichun Gao, Jasper F. Ooms, Rob Leurs and Henry F. Vischer

The histamine H_3 receptor (H_3R) is a presynaptic G protein-coupled receptor (GPCR) that plays a pivotal role in regulating the release of histamine and other neurotransmitters in the brain, making it a potential target for therapeutic interventions in neuronal disorders. The human H_3R gene encodes seven splice variants that conserve the seven transmembrane helical domains but vary in the length of intracellular loop 3 (ICL3) and/or the C-terminal tail. Despite that the ICL3/C tail of GPCR is important for G protein/Arrestin coupling, the comprehensive signaling transduction and functionality of these H_3R isoforms remain elusive.

To address this gap, we developed G protein and β-arrestin recruitment assays to assess the interaction of activated H_3R isoforms with their respective downstream effectors in real time by NanoBiT complementary technology. We found the H_3R-453, H_3R-415, and H_3R-413 isoforms were capable of recruiting the mini-G_i protein in a similar way as the canonical isoform H_3R-445 upon 10 μM histamine stimulation. Whereas the low amplitude response from the H_3R-373, H_3R-365 and H_3R-329 isoforms was related to their high constitutive activity. β-arrestin 2/1 recruitment to H_3R-445, H_3R-415, and H_3R-413 isoforms exhibited a sustained signal over time upon histamine stimulation, whereas H_3R-365 and H_3R-329 isoforms displayed a transient kinetical profile. However, H_3R-453 and H_3R-373 showed no β-arrestin2/1 recruitment upon histamine stimulation. In conclusion, our findings contribute to a more comprehensive understanding of H_3R and its isoform signaling. The insights may shed light on their potential roles as presynaptic receptors in modulating neurotransmitter release and neuronal function.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION IV – DRUG DESIGN & MOLECULAR TARGETING

REACHING BEYOND THE UV SPECTRUM – A PHARMACOLOGICAL PROFILE OF A RED-SHIFTED H_1R PHOTOCAGED LIGAND

Ivana Josimovic, Yang Zheng, Maikel Wijtmans, Henry Vischer, Rob Leurs

Photopharmacological modulation of GPCRs offers new ways to modify on-target activity of ligands in a spatio-temportal manner. One approach involves the introduction of a photosensitive moiety to a pharmacologically active compound, hereby blocking its activity (*photocaging*). The active compound can then be irreversibly *uncaged* with light. However, many available caged photoligands rely on UV light for uncaging, which can be damaging for *in vitro* and *in vivo* systems. Longer wavelengths are lower in energy and therefore provide better tissue penetration, hereby opening doors for more efficient real-time photo-modulation of ligand activity, while being less invasive for the system. We designed and synthetized an infrared-shifted photocaged histamine H_1R antagonist, VUF25549. The compound has more than 50 times lower H_1R affinity than its uncaged counterpart and has little to no activity in GPCR signalling assays. It can be uncaged in live-cell confocal imaging assays. Our research shows not only that H_1R antagonist photoligand activity can be modulated by light, but also highlights the potential of real-time on-target activity modulation of H_1R by light in living cells.

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TETRAZINE-BASED BUILDING BLOCKS FOR CLICK CHEMISTRY ON HISTAMINE H3 RECEPTOR LIGANDS

M. Stark, L. Leitzbach, H. Stark

Structural diversity in histamine H3 receptor (H3R) antagonists has dramatically increased within the last two decades. Nevertheless, Pitolisant stands out as a widely recognized reference compound, beeing the only approved H3R antagonist drug for sleep-related diseases. Increasing knowledge on the pharmacological profile as well as on potential therapeutic effects gave fresh impetus to multitargeting ligands for potentially improved therapy. The H3R pharmacophore on non-imidazole compounds can be combined in different ways with additional pharmacophoric elements while maintaining its H3R affinity. Click chemistry has been a versatile technique to combine different chemical motifs in an efficient way. Despite the widespread use of azide-alkyne 1,3-diploar cycloaddition, we have employed tetrazine-based compound development to new approaches for H3R ligands and easy chemical combinations. Notably, to the best of our knowledge, no H3R ligands are currently known to have the capability to engage in metal-free Click chemistry reactions, rendering them intriguing for *in vivo* applications. Here, we introduce the synthesis and biological evaluation of innovative ligands for the H3R featuring a methyl tetrazine moiety, designed to function as one partner of building blocks for Click chemistry. These novel compounds are characterized by a piperidinoalkyl unit connected to a *para*-substituted phenol. The tetrazine building block, effective for the inverse electron-demand Diels-Alder (IEDDA) Click reaction, is attached directly at the *para*-position or via alkyl phenol ethers with diverse aliphatic chain lengths. Throughout the course of synthesis, we also obtained and isolated the corresponding methyl dihydrotetrazines, subsequently subjecting them to H3R affinity testing. The radioactive displacement assay on H3R showed that all compounds featured K_i values in the lower nanomolar concentration range. This work presents a small series of novel H3R ligands exhibiting good H3R affinity and versatile capability to Click chemistry reactions via IEDDA for different building blocks with ring-strained dienophiles across various *in vitro* and *in vivo* applications in bioorthogonal applications for increased structural and functional diversity.

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ORAL PRESENTATIONS ABSTRACT

YOUNG INVESTIGATORS AWARDS

HISTAMINE RECEPTOR H_4 MODULATES THE EXPRESSION OF Na+/H+ (NHE)3 exchanger IN THE RENAL PROXIMAL TUBULAR CELL LINE HK-2

C. Gerbino, F. Foglietta, E. Benetti, AC. Rosa

In the proximal tubule, the 50% of sodium reabsorption is achieved by the Na^+/H^+ (NHE)3 exchanger. It plays a crucial role in the regulation of sodium and fluid balance, blood pressure homeostasis, and pathophysiology of hypertension. Moreover, NHE3 expression is dramatically increase in diabetic nephropathy and contributes to albuminuria. In a murine model of diabetic nephropathy, the histamine H_4 receptor antagonist JNJ39758979 prevented the oversexpression-induced of NHE3.
The aim of this study is to investigate whether histamine directly affect the functional expression of NHE3 in HK-2 cells, an in vitro model of renal proximal tubule.
Cells were exposed to histamine 0-1000nM for 0-48h and NHE3 gene and protein expression were evaluated by qPCR and Western blot. The activity of NHE3 was measured as intracellular pH (ipH) recovery after NH_4Cl loading by the BCECF-AM-based method. The receptor antagonists chlorpheniramine 10μM and JNJ7777,120 1μM were used to dissect the specific receptor contribute. The involvement of MAPK as signaling pathway downstream was also assessed.
Exposure to histamine induced a concentration-dependent NHE3 gene and protein transcription with a peak at 100nM from 16h followed by protein translation after 48h. Consistently at 48h histamine reduced the ipH recovery time, suggestive of an increased NHE3 activity. JNJ7777120 blunted both the activation and the expression of NHE3, while chlorpheniramine was effective only on NHE3 activity. Similar effects for histamine100nM were observed already at 60 min. The functional and the transcriptional effects were redundantly mediated by p38 and p42/44.
In conclusion histamine activating both H_1 and H_4 receptor regulates NHE3 activity in the proximal tubule with both early (60min) and late (48h) effects. These results strengthen the hypothesis of histamine as a target in kidney diseases where NHE3 could have a crucial role

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ORAL PRESENTATIONS ABSTRACT

YOUNG INVESTIGATORS AWARDS

MULTITARGETING COMPOUNDS FOR NEURODEGENERATIVE DISEASES: DUAL H3R/ChEs LIGANDS WITH CHELATING PROPERTIES

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The strategy for addressing multiple targets simultaneously stands as an advancement in the treatment of multifactorial diseases, such as neurodegenerative diseases, in which the progressive neuronal death leads to several dysfunctions, including the cognitive decline. The role of metal ion-catalyzed oxidative stress in the neuronal death is well established, as well as the involvement of acetylcholine and histamine on cognition.

Thus, the modulation of targets related to histaminergic and cholinergic neurotransmission, along with the antioxidant properties of chelating agents, may be an alternative in the treatment of these conditions. In this work, our aim was to synthesize and evaluate a set of compounds (**LINS05d**) combining histamine H_3 receptor (H_3R) antagonist, cholinesterases (ChEs) inhibitor and chelating properties in one molecule. The compounds were designed by overlapping structural features required for binding at H_3R and ChEs, also containing ion chelating groups. Eighteen compounds were obtained with satisfactory yields, which were tested in anti-AChE/BChE assays. Their affinities at the H_3R were assessed by binding assays and the chelating properties were evaluated spectrophotometrically using $Fe^{2+/3+}$, Cu^{2+} and Zn^{2+}. Compounds **613**, **313** and **433** were the most potent anti-ChEs in the series (pIC_{50} 4.4-4.9), while compounds **413**, **414** and **616** presented the highest affinity values for H_3R (pK_i 6.4-6.5). Isoquinoline and quinoline moieties led to higher affinity at H_3R, while the benzylpiperazine motif mostly contributed to the anti-ChE activity. The best chelating activity was obtained with isoquinoline derivatives. From the multitarget perspective, compounds **413** and **433** can be highlighted as the most promising. This study suggests that isoquinoline and benzylpiperazine moieties can provide the most interesting dual H_3R/ChE activity with chelating properties. The compounds herein reported represent interesting multitargeting prototypes for further studies.

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ORAL PRESENTATIONS ABSTRACT

YOUNG INVESTIGATORS AWARDS

VUF26063: A SECOND-GENERATION PHOTOSWITCHABLE LIGAND TO OPTICALLY CONTROL THE HISTAMINE H_3 RECEPTOR

Ivana Josimovic, Lars Binkhorst, Icaro Simon, Bas de Boer, Barbara Zarzycka, Iwan de Esch, Maikel Wijtmans, Henry Vischer, Rob Leurs

Photopharmacology uses light-sensitive ligands as tools to yield spatiotemporal control of protein activity and has been emerging in recent years in the field of G protein-coupled receptors (GPCRs). The histamine H_3 receptor (H_3R) is highly expressed in the central nervous system and has been identified as a potential target in diseases such as obesity, narcolepsy, Alzheimer's, and ADHD. Previously, our group published the first generation of azobenzene-based photoswitchable H_3R antagonists. In this research, we aimed to improve their photochemical properties by replacing the classical azobenzene with an arylazopyrazole photoswitchable moiety. Compared to first generation H_3R antagonists, key compound VUF26063 shows improved switching of 93% from photostationary state (PSS) PSS_{cis} to PSS_{trans} using a less damaging wavelength of 500 nm, instead of UV light at 430 nm. Improvements of pharmacological parameters include a nanomolar H_3R affinity (pK_i) for $VUF26063_{trans}$ and a ~50 fold affinity difference between isomers. In addition, arylazopyrazole-based H_3R antagonists show a ~13 fold inhibitory potency (pIC_{50}) difference between VUF26063 isomers in a histamine-induced PKA functional assay in HEK293T cells. Furthermore, VUF26063 can be repeatedly switched back and forward between isomers *in situ* within this assay.

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ORAL PRESENTATIONS ABSTRACT

YOUNG INVESTIGATORS AWARDS

DO HISTAMINERGIC NEURONS EXPRESS ADENOSINE 1 RECEPTORS? COMPARATIVE ANALYSIS WITH REFERENCE TO HIPPOCAMPAL DENTATE GYRUS GRANULAR CELLS

Lea Wegmann, Helmut L. Haas, Olga A. Sergeeva

Adenosine, the degradation product of ATP, is a sleep pressure factor playing an important role in homeostatic behavioral state control. The adenosine 1 receptor (A1R) is most sensitive to adenosine and reports sleep need to our brain. Controversial data were published during the last 18 years regarding hypothalamic histaminergic neurons (HN) controlling wakefulness. Recent reviews describe a particular role of HN in the action of adenosine, whereas we have not seen responses to adenosine in 13 rat HN in 2006. We analyzed single cell whole-transciptome data published recently and did not find A1R in any HN data bases.“Hipposeq" reported that among adenosine receptors only A1R is expressed by hippocampal dentate gyrus granular cells (DGgc) which can be identified by the expression of PDZd2. We selected these cells as positive control. We performed in parallel electrophysiology and PCR to identify HN and DGgc, to study responses to adenosine and expression of A1R in mouse brain slices. Quantitative PCR showed that hippocampus of adult mice expresses 6 times higher level of A1R compared to the posterior hypothalamus. In 70% of DGgc cells A1R was detected with single cell RT-PCR, whereas none of HN were A1R positive. In patch-clamp experiments one HN out of 22 (17 mice) was inhibited by adenosine in a DPCPX (A1R antagonist)-sensitive fashion, whereas 14 out of 16 DGgc were identified as A1R-positive by their pharmacology (FEPT $p<0.0001$). In primary dissociated cell cultures of hippocampus, inhibition of firing rate by adenosine was significantly stronger than in cultures made of posterior hypothalamus (MWT, $p<0.001$). We conclude that the vast majority of HN during waking (when these cells are firing) do not sense adenosine. This represents an important advantage for the allostatic regulation of waking: to sleep in the right time and place and to be awake as long as necessary during the fight or flight response.

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HALOPERIDOL, CLOZAPINE AND MIRTAZAPINE ARE FUNCTIONAL ANTAGONISTS AT HISTAMINE RECEPTORS IN HUMAN ATRIAL PREPARATIONS

J.M.A. Schlicht, B. Hofmann, U Gergs, J. Neumann

Psychiatric drugs have pleiotropic properties and are also antagonists at histamine receptors. Hence, we tested the hypotheses: firstly that haloperidol or clozapine antagonize H_2-histamine receptor-mediated positive inotropic effects in the human heart, secondly that mirtazapine antagonizes H_1-histamine receptor-mediated positive inotropic effects in the human heart.

To this end, we measured isometric force of contraction of human right atrial preparations (HAP), obtained during bypass surgery, in the organ bath. H_1 histamine receptors in HAP were examined by adding 0.4 µM propranolol and 100 µM cimetidine first. Afterwards, concentration response curves to histamine were established in HAP by applying histamine cumulatively from 10 nM to 100 µM. Then, in the continuous presence of 100 µM histamine, we noted negative inotropic effects (NIE) for additionally applied clozapine or haloperidol (1 µM, 3 µM and 10 µM each). The NIE of clozapine was more pronounced than the NIE for haloperidol. In some samples, pre-incubation with 1, 3, or 10 µM haloperidol or clozapine was performed followed by a concentration response curve for histamine. In this way clozapine but hardly haloperidol shifted the concentration response curve to histamine to the right, suggesting H_2-histamine receptor antagonism. In the presence of 0.4 propranolol, 100 µM cimetidine and 100 µM histamine, mirtazapine (1 µM, 3 µM and 10 µM) exerted a time dependent NIE ($n=4–6$, $p<0.05$) suggesting antagonism at H_1-histamine receptors in HAP.

These findings suggest a NIE of clozapine by antagonism at H_2-histamine receptors even in therapeutic doses (1.1 – 1.8 µM). However, haloperidol has lower therapeutic plasma concentrations (13 – 45 nM). Hence, effects will only be seen in accumulation or overdosage. Similarly, negative inotropic effects of mirtazapine through H_1-histamine receptor antagonism seem to start at therapeutic concentrations (151 – 301 nM), but are more distinct at higher concentrations.

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SCIENTIFIC SESSION V IN MEMORY OF PROF. PIER F. MANNAIONI –
HISTAMINE IN THE HEART & THE KIDNEY

CONTRACTILE EFFECTS OF HISTAMINE IN MICE OVEREXPRESSING H_1-HISTAMINE RECEPTORS AND H_2-HISTAMINE RECEPTORS IN THE ATRIUM

T.H. Pham, L.M. Rayo Abella, U. Kirchhefer, J. Neumann, U. Gergs

In the human heart, H_1- and H_2-histamine receptors are coexpressed. To get a comparable mouse model, we crossbred two mouse lines with cardiac myocytes-specific overexpression of either human H_1-histamine receptors (H_1-TG) or human H_2-histamine receptors (H_2-TG) to obtain double transgenic mice (H_1xH_2-TG) and compared them with wild type mice (WT). We measured force of contraction in isolated electrically stimulated left atrial preparations (LA) and spontaneously beating right atrial preparations (RA). In LA from WT, cumulatively applied histamine (1 nM – 30 µM) did not affect force of contraction. In LA from H_1xH_2-TG, low concentrations (30 nM – 1 µM) of histamine exerted a positive inotropic effect (PIE) whereas higher concentrations (3 µM, 10 µM, 30 µM) of histamine exerted a negative inotropic effect (NIE). The PIE of histamine in H_1xH_2-TG was antagonized by 10 µM of the H_2-histamine receptor antagonist cimetidine, the NIE of histamine in H_1xH_2-TG was antagonized by 1 µM of the H_1-histamine receptor antagonist mepyramine. Higher concentrations of histamine (3 µM, 10 µM) led to a negative chronotropic effect (NCE) in RA from H_1xH_2-TG that was antagonized by 1 µM mepyramine. Cumulatively applied dimaprit (1 nM – 10 µM), a H_2-histamine receptor agonist, exerted at 300 nM and higher concentrations only a PIE in LA from H_1xH_2-TG. Unexpectedly, 2-(2-Thiazolyl) ethyl amine (ThEA), a supposed H_1-histamine receptor agonist, led only to a PIE and PCE in LA or RA from H_1xH_2-TG, respectively. This controversy needs to be clarified through further experiments.
In summary, these data indicate that histamine first activates H_2-histamine receptors and then H_1-histamine receptors.
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TEMPERATURE SENSITIVE CONTRACTILE EFFECTS OF HISTAMINE IN MICE OVEREXPRESSING H_1-HISTAMINE RECEPTORS IN THE ATRIUM

P. Grundig, J. Neumann, U. Gergs

Histamine does not alter force of contraction in left atrial preparations from wild type mice (WT). However, histamine exerts an initial negative inotropic effect followed by a positive inotropic effect in isolated left atria of mice with cardiac-specific overexpression of human H_1-histamine receptors (H_1-TG). We tested the hypothesis whether the cardiac effects of histamine might be temperature sensitive. Hence, we measured under isometric conditions the effect of histamine on isolated atrial preparations under normothermia (37°C), hypothermia (24°C), and hyperthermia (42°C) in the organ bath. Additionally, the spontaneous beating rate and force of contraction were measured in the absence and presence of histamine. Atria were pretreated with 0.4 µM propranolol to exclude any β-adrenergic effects. At normothermia, histamine (1 µM) induced an increase in force of contraction in H_1-TG. The transient negative inotropic effect amounted to 14% ($n=5$, $p<0.05$). The relative increase in force in the biphasic effect was about 26%. Under hypothermia, a force increase of 185% ($n=5$, $p<0.05$) was observed. The relative increase in force in the biphasic effect was about 87%. However, under hyperthermia, histamine failed to raise the force of contraction. The negative inotropic effect was about 61% ($n=5$, $p<0.05$). The frequency at normothermia increases by 16%. Under hypothermia, there was a 105 % increase and under hyperthermia, a 60% increase in frequency.
In WT, there was a decrease in contractile force, in hypothermia and hyperthermia. In WT right atria under normothermia, we detected a 14% increase in beating rate, hypothermia exhibited a 182% increase, and hyperthermia showed a 26% increase.
In summary, there is a relation between temperature and force as well as beating rate in both TG and WT, which varies depending on the expression of H_1-histamine receptors. These effects may potentially have clinical applications.

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SCIENTIFIC SESSION V IN MEMORY OF PROF. PIER F. MANNAIONI –
HISTAMINE IN THE HEART & THE KIDNEY

DIFFERENT ROLE OF HISTAMINE H_1 AND H_4 RECEPTORS ON THE EXPRESSION OF AQUAPORIN (AQP)1 AND AQP7 IN THE HUMAN RENAL PROXIMAL TUBULE CELL LINE HK-2

M. Molino, F. Foglietta, A. Pini, A. C. Rosa

Aquaporins (AQPs) are proteins for bidirectional water transport, that regulate numerous processes such as urine formation and concentration. Among the isoforms present at the renal level, AQP1 and 7 are specifically expressed in the proximal tubule, where histamine receptors H_1 and H_4 are also expressed and contribute to reabsorption. Since histamine has already been described to regulate AQPs in nasal epithelial and in gastric adenocarcinoma cells through histamine H_1 and H_2 receptors respectively, we aimed to demonstrate its effect in influencing AQP1 and 7 expression in the proximal tubule.
Human proximal tubule cells (HK-2) were exposed to histamine (from 0 to 100 nM) for 0 to 24 h and the expression of AQP1-7 was evaluated by western blot analysis. Chlorpheniramine 1 µM and JNJ7777120 10 µM were used to dissect the histamine receptor subtype contribute.
Histamine exposure resulted in a downward trend in the AQP1 level and a significant increase in AQP7 expression in a time- and concentration-dependent manner. An increased expression of the glycosylated form- the functionally expressed one- of both AQP1 and AQP7 was also observed. To investigate the relative contribution of histamine H_1 and H_4 receptors in the AQP glycosylation process, the same experiments were repeated in the presence of the specific antagonists. The results clearly demonstrated that both H_1 and H_4 receptors are involved in the AQP7 glycosylation process, while only H_4 receptors affected the AQP1 post-translational modification.
The data suggest that histamine contributes to the dysregulation of renal resorption, a phenomenon underlying many diseases characterized by polyuria, affecting the AQPs expression on tubular epithelial cell membrane. However, further studies are needed to better understand how histamine influences the signaling pathways involved in AQP1 and 7 expression.

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SCIENTIFIC SESSION V IN MEMORY OF PROF. PIER F. MANNAIONI –
HISTAMINE IN THE HEART & THE KIDNEY

DIFFERENT ROLE OF HISTAMINE H_1 AND H_4 RECEPTORS ON THE EXPRESSION OF AQUAPORIN (AQP)1 AND AQP7 IN THE HUMAN RENAL PROXIMAL TUBULE CELL LINE HK-2

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INVESTIGATION OF THE CROSS-TALK BETWEEN HISTAMINE AND RENAL CLC CHLORIDE CHANNELS AND TRANSPORTERS IN A MOUSE MODEL OF DIABETIC NEPHROPATHY

E. Conte, P. Imbrici, C.E. Monreale, M. Pati, A.C. Rosa, A. Liantonio

Different lines of evidence point out to a potential direct contribution of histamine to the renal pathophysiology. In this context, histamine is emerging as a new mediator in diabetic nephropathy (DN), the most common complication in patients with diabetes mellitus, being able to exert a series of activities along nephron through the four subtypes of G-protein coupled receptors ($H_{1\text{-}4}$Rs). Accordingly, H_1R and H_4R are now considered promising drug targets, being H_1R antagonist bilastine and H_4R antagonist JNJ39758979 able to prevent the alteration of several indexes of DN-associated renal dysfunction. In view of the tubule-centric hypothesis of DN etiopathogenesis, renal ion channels and transporters could be involved in histamine receptor-mediated polyuria and proteinuria. In this regard, ClC-K1 and ClC-K2 chloride channels and ClC-5 Cl^-/H^+ antiport are proteins belonging to the ClC family involved in the reabsorption of salt and low molecular weight (LMW) proteins, showing an overlapping expression localization with $H_{1\text{-}4}$Rs in specific nephron segments. Thus, we here explored the potential cross-talk between these renal ClC proteins and histamine by using the streptozotocin-induced murine model of DN, showing histamine high levels, and by performing a gene and protein expression study. In line with polyuria and proteinuria characterizing DN, a reduction of ClC-K1, ClC-K2 and ClC-5 gene and protein expression was observed in kidney diabetic mice with respect to control mice. Preliminary results regarding the administration of JNJ39758979 and bilastine allowed to gain insight into the specific involvement of HRs in the regulation of ClC gene expression and indicated a key role for H_1R. Our findings revealed that renal ClC proteins can be considered new players in DN etiopathogenesis in relation to histamine-mediated kidney dysfunction.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

H_4 HISTAMINE RECEPTOR UPREGULATION AND INFLAMMATORY RESPONSES IN AN *IN VITRO* MODEL OF DIABETIC RETINOPATHY

Paul Chazot, M Hussan, S Grillo

Our knowledge of the underlying molecular mechanisms for the development of diabetic retinopathy is still evolving. Chronic exposure to hyperglycaemia and other causal risk factors leads to a cascade of biochemical and physiological changes that ultimately causes retinal dysfunction and microvascular damage. Müller cells are one of the primary glial cell types found in the retina, and since they are the only cells that span the entire width of the retina and have contact with both retinal blood vessels and retinal neurons, they are uniquely positioned to perform a variety of functions to maintain retinal function and homeostasis. It has been suggested that the breakdown of the blood retinal barrier is caused by improper localization of the dystrophin-Dp71 protein as deletion of the protein causes the protein to localize in the endfeet of Müller cells, thus causing extensive vascular leakage and oedema in the mouse retina. There is an increased histaminergic tone associated with diabetes, and emerging evidence also points to a role of histamine in the BBB breakdown that is associated with the pathogenesis of diabetic retinopathy and vascular permeability, the H_4 receptor is a potential target for therapeutic interventions in diabetic retinopathy.

In this study, we explored the presence and role of histamine and the histamine H_4 receptor, using immunofluorescence, calcium signalling ($Calcium_{green}$ probe), and TNFα ELISA methods. Immunofluorescence studies using HDC and H_4 receptor-specific antibodies in the human Müller cell line Mio-1, showed the presence of histamine decarboxylase (HDC) and the H_4 receptor in the cells, the latter specifically in the projections of the cells. This fits well with the observation, in the mouse retina, where H_4 receptor labelling was prominent in the end-feet of the Müller cells (see above). When the human Müller cells were grown in high glucose media (4500mg/L D-Glucose), the expression of the H_4 receptor appeared significantly more prominent compared to when the cells were grown in standard low glucose media. This is consistent with several studies that has implicated the role of histamine in diabetic conditions through upregulation of histamine in diabetic patients, and the histamine H_4R rodents, eg. kidney. Following administration of the selective H_4 receptor agonist VUF8430 (100 nM) to hyperglycaemic Mio cells, there is an increase in intracellular calcium, and TNFα, the former under baseline conditions, and the latter under inflammatory stress conditions (glyoxal, 2 μM). Additionally, when mice were fed a high fat diet (HFD) over 3 months, H_4R appeared to be increased in the inner plexiform layer of the retina. This suggests that HFD may increase the H_4R expression at the synaptic connections between bipolar cells and retinal ganglion cells. Overall, these data suggest the potential use of a H_4R antagonist drug in treating diabetic retinopathy.

Thanks Prof R Leurs for the sample of VUF8430.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

HISTAMINE STIMULATES CALCIUM-DEPENDENT TRANSMEMBRANE CATION CURRENT THROUGH H_1R-$G_{q/11}$ PATHWAY IN HUMAN RETINAL GLIAL MÜLLER CELLS

J. Strikland, R. Robson, I. Obara, M. Alsaqati, P.L. Chazot, P. Yarova and V. Telezhkin

Histamine is a ubiquitous autocoid that mediates a vast variety of physiological processes through the binding to one of its four G-protein coupled receptors (H_1R, H_2R, H_3R and H_4R). Using patch-clamp electrophysiology in conventional whole-cell configuration, and calcium (Ca^{2+}) imaging we found that histamine induced powerful conductance that was associated with augmented intracellular Ca^{2+} in human retinal glial Müller cells MIO-M1. Inhibition of the histamine effects with H_1R antagonist and phospholipase C inhibitor U73122 unveiled the signal transduction through $G_{q/11}$ cascade. Suppression of intracellular Ca^{2+} signal and transmembrane current with xestospongin C confirmed further involvement of Ca^{2+} release from endoplasmic reticulum (ER) through inositol triphosphate receptors (IP_3R), and passive depletion of ER stores with reticular Ca^{2+} adenosine triphosphatase (Ca^{2+} ATPase) inhibitor cyclopiazonic acid (CPA) and Ca^{2+} chelator BAPTA-AM verified store-dependence of the histamine signalling in human retinal glial Müller cells MIO-M1. Sensitivity of the histamine signals with nominal Ca^{2+} free solution and $NiCl_2$ highlighted importance of the extracellular Ca^{2+} influx, that was confirmed with 2APB the inhibitor of transient receptor potential channels, presumably melastatin (TRPM) subfamily. The expression of H_1R, TRPM7 and glial markers was confirmed using immunocytochemistry. Taken together, this study establishes a novel H_1R/TRPM7 mechanism that in human retinal glial Müller cells might play important role in retinopathies such as fluid accumulation in macular oedema and neovasculisation in diabetic retinopathy.

Authors would like to thank Prof. Astrid Limb (UCL) for kind gift of human retinal glial Müller cells MIO-M1cells.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

H_3R ANTAGONIST-CA INHIBITOR HYBRID COMPOUNDS IN A RABBIT MODEL OF TRANSIENT OCULAR HYPERTENSION

L. Lucarini, S. Villano, S. Sgambellone, S. Marri, E. Masini, A. Nocentini, C.T. Supuran, F. Iovane, M. Schultes, L. Leitzbach, T. Werner, H. Stark

Glaucoma is a leading cause of blindness, afflicting more than 60 million people worldwide with a discouraging estimate of 111 million in 2040. It is an optic neuropathy, characterized by an imbalance between production and outflow of aqueous humor (AH), generating an increase of intraocular pressure (IOP), the main risk factor of glaucoma. The histaminergic system plays an important role in the regulation of IOP through histamine H_3 receptors (H_3Rs) in the eye. Selective antagonists for H_3R increase the AH outflow, lowering IOP, and carbonic anhydrase inhibitors (CAI), such as dorzolamide (DRZ), can decrease IOP. Current pharmacological therapies are effective in reducing it, but not all the patients are responsive, and important side effects impair the compliance, accounting for the necessity of novel therapeutic approaches. The aim of the research was to evaluate the capability of a new set of H_3R antagonist-CAI hybrid compounds, acting at different biological levels, in controlling high IOP. A transient ocular hypertension (OHT) model in New Zealand White (NZW) rabbits was used to address the potential IOP-lowering ability of newly designed potential hybrid compounds ST-2525, ST-2558, ST-2559 and ST-2493, compared with ciproxifan and dorzolamide as reference drugs. The compounds were evaluated for inhibitory activities at human H_3R and on selected CA isoforms. The transient OHT model was induced by injection of 50 µL of 5% hypertonic saline into the vitreous. IOP was measured with a Pneumotonometer at baseline and 60, 120, and 240 min post treatment after OHT induction. The effects were maximal with ST-2525 (K_i<10 nM for hH_3R and hCA II and XII) at 60 min post-dose ($\Delta\Delta IOP_{60'}$= -6.0±0.9 mmHg, at 1%), maintaining its activity at 120 min ($\Delta\Delta IOP_{120'}$= -4.6±1.4 mmHg) and after 240 min ($\Delta\Delta IOP_{240'}$= -4.4±0.9 mmHg).
These results suggest that H_3R antagonist-CAI hybrid compounds contribute in IOP regulation with a long-lasting effect, and they could represent a future promising therapy for glaucoma.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

EVALUATION OF FIBROTIC MARKERS IN AN ANIMAL MODEL OF OCULAR HYPERTENSION TREATED WITH A HISTAMINE H3R/NO HYBRID COMPOUND

S. Sgambellone, S. Marri, S. Villano, A. Frank, D. Reiner, M. A. Khanfar, H. Stark, E. Masini, L. Lucarini

Glaucoma is a degenerative ocular disease characterized by a degeneration of ciliary processes and trabecular meshwork (TM). This malfunction triggers a series of cascading events that can lead to progressive vision loss and finally blindness.
Fibrosis is a common pathological feature in many chronic diseases. It is a tissue repair response manifested by excessive deposition of extracellular matrix (ECM) components such as collagen and fibronectin. ECM synthesis and degradation are tightly regulated processes that play a critical role in maintaining TM integrity and a dysregulation of these, can lead to fibrosis and impaired TM function. Severe fibrosis in the TM can lead to continuous abnormal ECM accumulation and distortion of the TM framework, resulting in increased resistance to aqueous humor outflow and elevated intraocular pressure (IOP).
Histamine receptors are known to have an important role in glaucoma, and histamine H_3 receptor (H_3R) antagonists have been shown to be effective in ameliorating the glaucomatous condition. Based on this evidence this research aimed to evaluate whether a H_3R antagonist hybrid compound could reverse the fibrotic process in a chronic ocular hypertension (OHT) model. The chronic OHT was carried out on New Zealand White rabbits by a single injection of 100 µl of Carbomer 0.25% (Siccafluid, Farmila THEA Pharmaceutical) in the anterior chamber of the eye resulting in ocular hypertension that persisted for 14 days. The animals were treated with a H_3R antagonist/NO donor hybrid compound ST-1989 and its reference drugs, Ciproxifan and Molsidomine, both administered alone and in combination. In ocular tissues, the TGF-β signaling pathway was assessed by determining TGF-β1 levels (ELISA KIT),
SMAD3, P-SMAD3 and α-SMA via Western blot and immunofluorescence analysis.
These preliminary results demonstrate that this compound reduces fibrotic markers confirming that it could represent a good strategy for the therapeutic treatment of glaucoma.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

PHOTOSWITHCHABLE COMPOUNDS IN A TRANSIENT OCULAR HYPERTENSION (OHT) MODEL IN NEW ZEALAND WHITE (NZW) RABBITS

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Glaucoma, a pathology involving retinal ganglion cells (RGCs), is an urgent public health disease. According to the World Health Organization (WHO), glaucoma is the second leading cause of blindness. This pathology is mainly caused by high intraocular pressure (IOP) caused by a disequilibrium between the production and outflow of aqueous humor (AH). Three subtypes of β-ARs (β1, β2, and β3) have been identified with different distributions among ocular tissues. β-ARs are an established target in the treatment of glaucoma. Even, phosphodiesterase type 5 (PDE5) is a key enzyme involved in the regulation of cGMP-specific signaling pathways in normal physiological processes, such as smooth muscle contraction and relaxation. For this reason, inhibition of PDE5 can alter pathophysiological conditions associated with low cGMP level in tissues. We studied two different types of photswitchable compounds: the β2 blockers (VUF25474 cis and trans) compared to propranolol and the PDE5 inhibitors (VUF25334 cis and trans) compared to sildenafil. Photoswitches are molecules that undergo structural changes in response to light irradiation. A transient ocular hypertension (OHT) model in New Zealand White (NZW) rabbits was used to study the potential IOP-lowering ability of the photoswichable compounds. This model was induced by injection of 50 µL of 5% hypertonic saline into the vitreous. IOP was measured with Pneumatonometer at baseline and 60, 120 and 240 min post treatment after transient OHT induction. The effects were maximal with VUF25474 cis 0.1% and with VUF25334 0.05% cis at 60 min post-dose. Concerning VUF25334 the IOP remained stable at 120 min post-dose and decayed thereafter to reach baseline values at 240 min. These results exhibited that photoswitchable compounds provided a long-lasting effect in IOP regulation, and they could be a future promising treatment for glaucoma. A novel recent approach represents an *in vitro* model to study these compounds with trabecular meshwork (TM) cells, that were treated with different concentrations of VUF25474 and VUF25334 in order to study histamine receptor signalling.

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SCIENTIFIC SESSION VI – HISTAMINE IN THE EYE

STUDY OF microRNA INVOLVED IN OCULAR ISCHEMIA PATHWAY

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Ocular ischemia is a pathological condition consisting in a restriction of blood flow related to various diseases, such as diabetic retinopathy or glaucoma.
At retinal level, ischemia alters the homeostatic balance between angiogenic and angiogenetic factors that limits aqueous humour outflow into the anterior chamber of the eye, resulting in increased IOP. An impairment of optic nerve head (ONH) vascular perfusion contributes to progressive depletion of retinal ganglion cells (RGCs) and optic nerve degeneration. In glaucomatous disease, the continuous IOP oscillation causes repeated ischemia/reperfusion (I/R) phenomena producing reactive oxygen species (ROS) that enhance tissue injury. The histaminergic system plays an important role in the maintenance of ocular vascular tone. Histamine H_3 receptors (H_3R) antagonists are known to be effective in ameliorating ocular vascular performance and preventing RGCs death. Nitric oxide (NO) is a pivotal molecule for the regulation of blood flow; in fact, NO donors are used for the treatment of several ocular diseases. MicroRNAs (miRNA) are implicated in oxidative stress, hypoxia and inflammation regulation. The aim of this research is to evaluate the modulation of histamine receptors and miRNA expression in ocular ischemia. The I/R model was carried-out in New Zealand White Rabbits by repeated injections of ET-1, twice a week for 6 weeks. Animals were treated with vehicle or ST-1989 1%, a histamine H_3 antagonist-NO donor hybrid compound, *bid* for 4 weeks. On ocular tissues, we evaluated miR-29b, that is involved in trabecular meshwork (TM) extracellular matrix (ECM) deposition through NF-Kb and TGF-β2 regulation pathway, miR-182, that acts as anti-oxidative and anti-apoptotic factor, miR-210 and miR-21, that are hypoxia-induced miRNAs.
The different expressions of these miRNAs confirm their involvement in ocular ischemia signalling pathways. Therefore, they can be used as a versatile tool for disease detection and may also be promising therapeutic targets.

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HISTAMINE UPREGULATES THE EXPRESSION OF MMP12 IN HUMAN M2 MACROPHAGES

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Beyond Th2 cells and various immune cells, M2 macrophages have been identified in lesional skin of atopic dermatitis (AD) indicating their involvement in the disease's underlying mechanisms. MMP12, a matrix-degrading enzyme, which is predominantly produced by macrophages, is increased in skin lesions of AD patients.

Our aim was to investigate the expression of MMP12 in M2 macrophages from healthy individuals and AD patients in response to Th2 cytokines and histamine. Additionally, macrophages from dupilumab-treated AD patients were analysed to assess the influence of Th2 cytokine on MMP12 expression ex vivo.

The expression of MMP12 mRNA in lesional skin of AD was evaluated at the single cell level through RNA sequencing (scRNA-seq) published previously in detail.

M2 macrophages derived from both healthy donors and AD patients were stimulated with IL-4 or IL-13, with histamine or specific histamine receptor agonists (H1R, H2R, H4R) either during or after differentiation. MMP12 mRNA expression was analysed using quantitative PCR. MMP12 concentrations in cell supernatants were measured via ELISA.

ScRNA seq analysis identified macrophages as the primary producers of MMP12 in lesional AD skin. In vitro, both MMP12 mRNA and protein expression were significantly increased in monocytes during differentiation to M2 macrophages in the presence of histamine, of Th2 cytokines or of Th2 cytokines in combination with histamine.

In M2 macrophages obtained from dupilumab-treated AD patients, the upregulation of MMP12 expression by IL-4 and IL-13 was attenuated.

Our findings reveal a novel mechanism by which Th2 cytokines and histamine regulate MMP12 expression, which may affect skin barrier homeostasis in AD.

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ORAL PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VII – HISTAMINE IN INFLAMMATION AND IMMUNITY

ASSESSING THE ANTHELMINTIC POTENTIAL OF ANTIHISTAMINES H_1 AGAINST *ANGIOSTRONGYLUS CANTONENSIS*

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Angiostrongylus cantonensis is a nematode that develops in molluscs and infects a variety of vertebrates, mainly rodents. Nonetheless, this parasite may cause human infection (angiostrongyliasis) after ingestion of contaminated food. After infection, the larvae migrate to the CNS, leading to eosinophilic meningitis, causing fever, headache and meningismus. To date, there is no specific treatment against angiostrongyliasis and therefore the search for therapeutic agents is needed. Considering the neglected status of worm infections on drug discovery programs, drug repurposing is an option to accelerate the search for drugs against helminthiasis, being this worm an interesting model for drug discovery against helmints. Since antihistamines were already reported as potential agents against a variety of parasites, including *Trypanosoma cruzi*, *Leishmania infantum* and also *Schistosoma mansoni*, in this work a screening of clinically available antihistamines was performed. A set of 21 anti-H_1 antihistamines commercially obtained were screened against first-stage larvae (L1) of *A. cantonensis* (n = 100 larvae/well), isolated from the feces of infected rats through Rugay's method. The althelmintic activity was measured by standardized score (effect >60%): 1 (immotile), 2 (intermittent shaking of the head or tail region), 3 (sluggish and motile), 4 (highly active and motile). Anthelmintic drugs ivermectin and albendazole were used as standard drugs. The results showed that four drugs (cinnarizine, desloratadine, promethazine and rupatadine) were active against the parasite, being promethazine the most potent (IC_{50} 31.6 µM). The activity has no direct correlation to the affinity to histamine receptor, but other mechanisms (affinity to cholinergic receptors or histamine-response proteins) may be involved. This is the first report of the activity of antihistamines against *A. cantonensis*, an important contribution to the search of novel agents active against nematodes.

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FLASH PRESENTATIONS ABSTRACT

SCIENTIFIC SESSION VII – HISTAMINE IN INFLAMMATION AND IMMUNITY

CYCLOPHOSPHAMIDE AUGUMENTS HUMORAL IMMUNITY AND DRUG RESISTANCE AGAINST L-ASPARAGINASE

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L-Asparaginase (ASNase), a key drug in the treatment of childhood acute lymphoblastic leukemia, often causes allergic reactions and drug resistance. However, when mice were sensitized with ASNase alone, the allergic response as well as IgE production were lower than those induced by ovalbumin. In the present study, among the drugs concomitantly used with ASNase, we examined the effect of cyclophosphamide (CY) on the immune response to ASNase.

Male BALB/c mice were intraperitoneally sensitized by ASNase and alum. CY was intraperitoneally injected 2-day prior to ASNase sensitization. Allergic skin reaction to ASNase was evaluated by measuring ear thickness after intradermal challenge of ASNase into the ear lobe. Total IgE level and ASNase activity in the sera were measured. RBL-2H3 cells were sensitized by the sera of immunized mice and the cells were stimulated by ASNase to determine β-hexosaminidase (β-Hex) release. Spleen cells of immunized mice were cultured for 48 hrs with concanavalin A and cytokines in the medium were measured using a Bio-Plex Th1/Th2 assay kit.

ASNase sensitization induced ear edema and increased serum IgE levels in mice. CY at 150 mg/kg augmented these responses. CY at 300 mg/kg increased serum IgE levels, but decreased ear edema and serum ASNase activity. Sera of CY 150 mg/kg-treated mice induced higher β-Hex release from RBL-2H3 cells than normal anti-ASNase sera, though those of CY 300 mg/kg-treated mice did not induce β -Hex release. After removing IgG from the sera of CY 300 mg/kg-treated mice, β -Hex release became higher than normally sensitized mice. ASNase sensitization induced a Th2-biased immune response, and the addition of CY further enhanced the Th2-biasd response in a dose-dependent manner.

CY administration enhanced Th2-biased immune responses and increased IgE and IgG production in the ASNase-sensitized mice. These findings suggest that CY may play a role in the development of ASNase allergy and drug resistance.

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EHRS International Anthem

Mel.Polly Waddle Doodle

CHORUS:

For it ś mine, for it ś 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 ŕe 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

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.

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 ŕe 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 ś 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.

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 ŕe in Germany to hear
How our Frido with Histamine can play.
And to Durham we went then / in the year two thousand ten.
There with Paul near Cathedral did we stay.

27. 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!
Then in **Turin** twenty four / the meetings will be more
And our Histamine will live for many years

CHORUS