THE ANOREXIANT EFFECT OF OLEOYLETHANOLAMIDE IS MODULATED

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The European Histamine Research Society 41st Annual Meeting, May 2–5, 2012 Belfast, Northern Ireland

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Meeting report of the European Histamine Research Society

G. Sturman

Last year's meeting in Sochi, Russia was the most eastern meeting we have ever held whilst this year's meeting (the 41st meeting of the European Histamine Research Society) was the most westerly one. We met in Belfast, Northern Ireland at the kind invitation of Professor Madeleine Ennis of Queen's University Belfast. This was the second joint meeting with COST Action 0806—see following article. These meetings took place in the Hilton Hotel, Belfast (2nd–5th May 2012). This was the first time we have ever met in Northern Ireland and we were situated right in the centre of Belfast beside the River Lagan and not far from the famous Harland and Wolff shipyards and the newly opened Titanic Exhibition Museum.

This year over 130 delegates attended representing 29 countries (most from the European countries, especially the UK but also from North and South America, Middle East, Australia and Japan). Some of the regular EHRS attendees could not attend and they were missed but a big welcome was given to all the new visitors, who we hope will return to future meetings. Most delegates arrived during the Wednesday. As usual the Council held their meeting late afternoon. Then there was a meeting for the COST BM 0806 WG 1 before our Welcome Reception; a delightful buffet with plenty of wine and fruit juices to drink. Old friends were greeted again whilst new ones were made.

On the Thursday, we began the meeting with the Opening Ceremony. We started with an introduction from our host Madeleine Ennis who informed us that 'There were no strangers present—just friends we have not yet met!' Then the Pro-Vice Chancellor for Research and Postgraduates at Queen's University Belfast, Professor James McElnay welcomed us to QUB and Northern Ireland. He spoke about the beginning of QUB in 1845 and gave an overview of the history and facilities within this University. Then our president, Anita Sydbom gave her welcoming talk, which centred round Irish folk customs, especially the folk dances and costumes. She then outlined the programme of this year's meeting. She then presented the student bursaries; certificates and cheques (each for €500) to nine student members. The society is very grateful to the El-Sayed Assem family for sponsoring 2 student bursaries while the G.B. West Memorial Trust gave another and the remaining six were from our society. This was followed by the presentation of certificates to 16 COST BM0806 Early Stage Cost researchers. Then there was the presentation of Honorary Membership to two of our very special members; first to Professor Zsuzsanna Huszti (Budapest, Hungary) and then to Professor Helmut Haas (Dusseldorf, Germany). Anita gave the laudation to Zsuzsanna and Patrizio Blandina (Italy) to Helmut. Both Zsuzsanna Huszti and Helmut Haas have made significant contributions to our society in many different ways over the years. They were presented with a certificate beautifully written in Latin and sporting the society's official seal. Then we heard the first of our plenary lectures. This was given by Holger Stark (Germany) and was entitled 'Histamine H_4 receptor—ligand, binding and activation'. Then at the start of our coffee break, we had a group photograph taken. This was followed by the Walter Schunack Memorial Symposium in which Holger Stark started by giving an appreciation to Walter. This was followed by 4 oral presentations on the chemical aspects of histamine receptor ligands. All the presenters gave very positive references to Walter, his work and support and also to his wonderful sense of humour. Continuing our tradition, each oral session at this meeting was chaired by an experience scientist as well as a young EHRS member so that they could get experience of chairing oral sessions.

Then we quickly moved outside to get the coaches for our outing. We drove along the northern side of the Belfast Lough to Carrickfergus Castle, where we stopped to explore this 800 year old castle. Participants could be seen climbing up on the battlements and visiting the various castle rooms. Then we continued along the Causeway Coastal route which hugs the narrow strip of coastline between the sea and the high cliffs. We passed through some picturesque towns and villages and saw some spectacular scenery, even sighting Scotland on the horizon as well as Raithin Island. Finally we reached Northern Ireland's most famous World Heritage Site, the Giant's Causeway, which was formed over 60 million years ago, when molten lava cooled suddenly on contact with water producing stone 'benzene ring' columns! Most people made their way down to actually clamber over the Giant's Causeway. Then after warming ourselves up with hot drinks we continued inland to Ballymena and the Galgorm Hotel. Here we were made very welcome and had a wonderful evening meal in this spectacular manor house. During our dinner, we were entertained by 2 talented Irish musicians from the band Pure Blarney singing and playing

the guitar, tin whistle and bagpipes. We all agreed that it was an excellent end to the first day of our meeting.

Friday began with the G.B. West lecture which was given by Peter Bradding (Leicester University, UK) and was entitled 'Interactions between mast cells and structural airway cells in the pathogenesis of asthma'. This was followed by poster sessions on 'Histamine and the Nervous System' and 'Mast cells, Metabolism and Chemistry'. Then we listened to three lectures on 'Novel highlights on mast cell functions' (COST BM 1007). After lunch, like last year, we held a Round Table which was entitled 'What's new in H_4 research? (COST BM 0806). Robin Thurmond (USA) lead a panel of histaminologists which included Elena Rivera (Argentina), Paul Chazot (UK), Ralph Gutzmer (Germany) and Hoger Stark (Germany) and needless to say this resulted in lively discussions. After the afternoon break, there was an oral session on 'Mast cells and Inflammation' followed by another one on 'Clinical aspects of the H_4 receptor' (COST BM0806). Then the WG3 COST Action BM0806 took place. After our evening meal, we took part in some Irish Whiskey Tasting. We were informed that whiskey was the 'Water of Life' and that Irish whiskey differed from Scotch as it was distilled three times rather than just twice. This thus gives Irish whiskey a much smoother taste. As predicted, there was much fun involved with this activity.

Our final plenary lecture by Beatrice Passini (Florence, Italy) started our Saturday's meeting. Her lecture was entitled 'H_4R and neuroinflammation: insights from mouse experimental autoimmune encephalomyelitis' and she gave a very interesting lecture despite the projector not showing the true colours of her histopathology results. This lecture was followed by an oral session on 'Histamine and the Nervous System'. After our coffee break, we viewed the poster presentations on 'Histamine—H_4 receptor' and also 'Clinical aspects and cellular studies'.

Throughout the meeting the poster committee had been working very hard and as usual had a difficult task in identifying winning posters for the poster competition. Eventually first prize was given to F. Jantzen et al. from Hannover, Germany, second to S. Micallef et al., from Dublin, Ireland and third prize to L. Kay et al., from Sheffield, UK.

The final oral session of our meeting was to listen to our younger members (PhD students or not more than 3 year's post-doctoral research) give their presentations for the EHRS Young Investigators Award. Regrettably one student had withdrawn at the last minute. It was another difficult task for the judges in differentiating between these four excellent presentations. The winner was Gustavo Provensi (Florence, Italy) with Naresha Saligrama (Vermont, USA) and Tomas Perecko (Bratislava, Slovakia) sharing joint second place and Yan Zhao (Lyon, France) being highly commended. To summarise the COST Action BM0806 work, Katherine Tiligada (Athens, Greece) told us about the 'Progress and Limitations in H_4R research.' Then we held our General Assembly. According to our statutes, a new Council had to be elected and after a ballot, Nick Carruthers (USA), Jian-Sheng Lin (France), Beatrice Passani (Italy) and Astrid Sasse (Ireland) were duly elected to serve on our Council. Additionally Anita's second and last term of office as President has now finished. Paul Chazot (Durham, UK) was unanimously elected as our new President. Many thanks were given to Anita for all her hard work to the society over the last 6 years. Our meeting ended with its traditional excellent Farewell Dinner followed by our award ceremony. The certificates and prizes were given out. Then as usual we had our singing session, beginning with "Anita's Thank You Song" (sung to the tune of 'It's a long way to Tipperary') as a big thank you to Madeleine and her team for the excellent meeting, and then we sung our EHRS Anthem. Afterwards 'Haste to the Wedding' played for us and we had a go at some Irish Ceilidh dancing—great fun was had by all.

We all agreed that the Northern Irish team, especially Madeleine had hosted a very well organised meeting with excellent science. Our thanks were given to all of them. The next meeting will be held in Lodz, Poland (8–11 May, 2013) at the kind invitation of Agnieszka Fogel.

Participants of the 2012 meeting of the EHRS

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Honorary Memberships in the European Histamine Research Society

P. Blandina, A. Sydbom, H. Haas, G. Sturman

There are three types of membership in the EHRS: ordinary, corporate and affiliated, and honorary (life). The highest award is that of honorary membership which is only given to very special people. To obtain honorary membership, the person has to be elected by over two-thirds of the ordinary members at the General Assembly. There are only 10 current Honorary Members of the Society; Madeleine Ennis, Robin Ganellin, Wilfried Lorenz, Piero Mannaioni, Bruno Mondovi, Fred Pearce, Henk Timmerman, Ingrid Olhagen-Uvnäs, Takehiko Watanabe and Jean West. Our former Honorary Members include Sir James Black, Franc Erjavec, Jack Peter Green, Czeslaw Maslinski, Wolfgang Schmutzler, Walter Schunack, Börje Uvnäs and Geoff West. At this meeting, the society awarded Honorary Membership to two special people who have both contributed significantly to the EHRS; they are Professor Zsuzsanna Husti and Professor Helmut Haas.

The oration for Professor Zsuzsanna Husti was given by our President, Anita Sydbom. Zsuzsanna Huszti, of the famous Semmelweis University in Budapest, Hungary, was born on the 16th of March 1936 in Szeged. In 1954 she entered the University of Szeged in the faculty of Natural Sciences where she studied chemistry. After getting her diploma in chemistry 1959, she worked as a research assistant at the Central Research Laboratory of the Szent-Györgyi Albert Medical School in Szeged. In 1962, Zsuzsanna started her doctorate work in biochemistry at the Eötvös József University, Budapest. At that time she also had a position as clinical chemist in the Central Hospital, Gyula, and as a research assistant at the Department of Pharmacology in the Drug Research Institute, Budapest. From 1971 to 1981 she was head of the Radioactive Laboratory at the Department of Biochemistry, Drug Research Institute, Budapest. From 1997 she became the head of the Neurobiobiology Unit in the Department of Pharmacodynamics, Semmelweis University of Medicine, Budapest.

Although her first publication was in 1963, her first one on histamine was in 1967 and her Ph.D. thesis (1972) was entitled "*Heterogeneity of monoamine oxidase in rat brain*". This was followed by postdoctoral position in the Department of Neurobiology, at McGill University, Montreal, Canada working with Professor Theodore L. Sourkes. This resulted in a fruitful collaboration and many papers about histidine metabolism. In 1994 Zsuzsanna produced a second thesis; this time for Doctor of Biological Science degree. This thesis was entitled "*Histamine in the nervous system: regulation of histamine synthesis and function in the brain*". She has published over 75 publications; most are concerned with histamine. She is/was a member of several scientific societies, namely the Hungarian Society of Biochemistry, International Society for Neurochemistry (ISN), Hungarian Pharmacological Society, European Society for Neurochemistry (ESN), International Brain Research (IBRO) and New York Academy of Sciences plus our society.

Zsuszanna Huszti has had a long standing connection with the EHRS. She was invited by Professor Maslinski, who had read her articles, to the very first informal meeting of the 'Histamine Club' held in Lodz, Poland 1971. After this meeting she has visited 36 EHRS meetings in total. She was the EHRS National Secretary for Hungary from 1972 to 2000. She gave an EHRS Invited Lecturer at the meeting in Eger (2004) with the title: *Histamine uptake by non-neuronal cells of the brain: properties and function.* She brilliantly organized two unforgettable EHRS meetings in Hungary, the 9th EHRS Meeting 1980 in Visegrad and the 23rd EHRS Meeting 1994 in Budapest. Many pictures from the different EHRS meeting can be seen under Previous Meetings on our website http://www.EHRS.org.uk. Zsuzsanna Huszti has for many years been devoted to our society, interested in science and young researchers and always is putting interesting questions to the speakers at our meetings. So with this it is a great honour to present the EHRS Honourary Membership to Zsuzsanna Huszti.

The oration for Professor Helmut Haas was given by Professor Patrizio Blandina. Helmut grew up in the south-west corner of Germany. After naval military service he studied medicine in Basel, Switzerland and Freiburg, Germany, where he obtained his M.D. (1968) for a dissertation on aphasia. Then he worked in the Psychiatric and Neurological University—Clinics in Basel before taking a postdoctoral position in the Department of Pharmacology, Cambridge, England. This was followed by a number of years as head of the Neurophysiology-Laboratory in the Neurosurgery-Department Zürich and visiting Professorships on sleep research at Harvard-University in 1986 and 1989. In 1987 Helmut became Professor and Head of the Department of Biophysics at Johannes-Gutenberg-

Universität Mainz, Germany; in 1991 he took the Chair of Neurophysiology at Heinrich-Heine-Universität Düsseldorf.

In 1971 Helmut began to work on histamine in the brainstem. He has since devoted much of his scientific life to this fascinating field characterizing histamine actions in the brain (e.g. Nature 1975, 1983, Neuron 1993) and later the properties of histaminergic neurons in the tuberomamillary nucleus using mainly electrophysiological methods. He has published over 200 peer-reviewed papers and serves on the editorial boards of several journals. His research was financed by the National Institutions in Switzerland and Germany and the Human Frontiers Science Programme (network mechanisms of memory trace formation) as well as the European Community (Consortium "food, mood and sleep" on orexins). Helmut, besides being a regular attendee of our meetings, hosted the 33rd EHRS meeting in 2004 near Düsseldorf. He is now, retired but not tired, cooperating with his wife (Prof. Olga Sergeeva) on new challenges of the histaminergic system in the brain. Helmut truly deserves the accolade of being elected an Honorary Member of this society.

Helmut Haas, Anita Sydbom (President) amd Zsuzsanna Huszti at the Farewell Dinner

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Meeting Report of the Cost Action BM0806 '*Recent advances in histamine receptor H_4R research*'

E. Tiligada

The main 2012 meeting of COST Action BM0806 '*Recent advances in histamine receptor H_4R research*' was hosted by Madeleine Ennis (Secretary of the Action, The Queen's University of Belfast, UK) and took place jointly with the 41st Annual EHRS conference held in the Hilton Belfast hotel, UK. The key objective of the Action BM0806 is to foster a multidisciplinary approach to histamine H_4 receptor (H_4R) research and to focus on the current state of play pertaining to the basic understanding and the huge therapeutic potential of this new drug target. The Action builds up strong European interdisciplinary links among more than 150 senior and young scientists from at least 40 research groups based on 20 COST (AT, CH, CZ, DE, DK, EL, ES, FI, FR, HU, IE, IL, IT, LT, NL, PL, SE, SI, SK, UK) and 4 non-COST (AR, NZ, US, RU) countries, who actively participate in the 4 mutually interlocking working Groups (WG) of the Action.

H_4R is the most recently identified histamine receptor subtype, which is primarily linked to immunomodulation and to a variety of inflammatory disorders. The development and the evaluation of selective H_4R ligands aim to promote the translational perspective in this field of research. COST Action BM0806 has a firm foundation for managing research into the H_4R function and therapeutic potential due to the combined efforts of scientists from multiple disciplines including biochemistry, molecular and cellular biology, genetics, chemistry, bioinformatics, pharmacology and medicine. Members of the COST Action are dominating the publications in this area, thus making Europe the leader in the field. This joint conference enhanced the previously established links with the EHRS, while the rapid and productive result dissemination and the direct exchange of ideas will enable the expert teams to work more efficiently towards the potential exploitation of this new drug target. Additionally, a delegate from Japan, the major non-COST country where H_4R research outside BM0806 is pursued, applied to join the Action. Importantly, the training of Early Stage Researchers (ESRs) in all aspects of H_4R research was broadened and their mobility by using the Short-Term Scientific Mission (STSM) COST tool was stimulated and encouraged during this joint activity.

The COST conference attracted 58 Action members from 22 member countries and comprised 3 COST Plenary Lectures, 2 COST sessions with 13 oral presentations and 14 peer-reviewed posters (5 related to STSMs), a round table session, a guest symposium from members of the COST Action BM1007, which concentrates on mast cell and basophil function, a BM0806 Management Committee meeting and WG1, 2, 3 & 4 meetings. In keeping with the tradition started at the Durham meeting in 2010, symposia and poster sessions were nearly all co-chaired by ESRs together with senior scientists. Sixteen ESRs received awards for their contribution to H_4R research, while the winner and the 2nd prize winner in the poster competition of this Joint meeting were both Action member ESRs (Franziska Jantzen, Hannover Medical School, DE; Stephanie Micallef, Trinity College Dublin, IE). In the Young Investigator Award, Tomáš Perečko (Slovak Academy of Sciences, SK) received joint 2nd prize as a result of the work that he performed during a COST BM0806 STSM.

An elegant description of H_4R ligands and the approaches for lead development was provided by Holger Stark (Goethe University, Frankfurt, DE). The data on the characterization of the H_4R binding pocket by molecular dynamics simulations and the binding properties of H_4R agonists, inverse agonists and neutral antagonists will be of immense use to the members of the COST Action. New information was provided by Beatrice Passani (University of Florence, IT) on the involvement of the H_4R in neuroinflammation and in particular in a mouse model of multiple sclerosis. Strategies aimed at interfering with the histamine axis may have relevance in the therapy of autoimmune diseases of the central nervous system (CNS) as histamine may determine a shift in T helper cell subpopulation, influence migration of lymphocytes and myeloid cells during CNS invasion, interfere with antigen presentation at the immune synapse level and determine variations in normal neuronal functions. During the guest symposium of the COST Action BM1007, Uli Blank (Inserm U699, FR) proposed the late signalling events in mast cell degranulation as a novel target for the development of drugs to block mediator release; Gunnar Nilsson (Karolinska Institutet, SE) reported on the secretion of large variety of oxylipins by mast cells and Francesca Levi-Schaffer (The Hebrew University of Jerusalem, IL) described the interactions between mast cells and eosinophils, which serve to perpetuate the inflammatory response.

The following highlights of the COST Action BM0806 presentations show the advancements on H_4R research. Rob Thurmond (Johnson & Johnson, US) offered insights on the interaction between H_4R and TLR activation in vivo, which can drive inflammatory responses. Arianna Rosa (ESR, University of Turin, IT) suggested a novel use for H_4R blockers in pulmonary fibrosis and also showed that the H_4R expressed in the kidney is up-regulated in diabetic male rats. Tomáš Perečko provided novel information on the presence of H_4Rs in human neutrophils and on their involvement in the inhibition of neutrophil degranulation. Interestingly, Maristella Adami (University of Parma, IT) reported on the effective inhibition of carrageenan-induced oedema by H_4R agonists rather than antagonists. Spyridon Chalkiadakis (ESR, University of Athens Medical School, EL) provided further evidence on the putative constitutive H_4R-mediated automodulatory histamine function in the rat conjunctiva using novel 2,4-diaminopyrimidine derivatives targeting the receptor. The presence of H_4Rs on peptidergic Aδ fibres (CGRP) and C-fibres (substance P) reported by Mwape Katebe (ESR, University of Durham, UK) could have implications not only in chronic pain but also in chronic cough, while evidence provided by Diego Martinel Lamas (ESR, University of Buenos Aires, AR) pointed to the potential therapeutic use for H_4R agonists in the treatment of breast cancer. Novel approaches to H_4R research were presented by Gniewomir Latacz (ESR, Collegium Medicum Jagiellonian University, PL) who found that some N-methylpiperazine derivatives influenced the motility of the promoter of *PTEN*, one of the most frequently mutated tumor suppressor genes in human cancer as well as by Anwar Rayan (GeneArrest, IL) who described the methods used to alter H_4R gene expression by novel and highly specific molecules which bind tightly and selectively to predetermined sequences in the major groove of double stranded DNA. Hubert Schwelberger (Medical University Innsbruck, AT) provided information on the progress of the *histamine methods and tools database* (HMTD), which will be of immense use to all working in the field. Finally, the Action Chair Katerina Tiligada (University of Athens Medical School, EL) presented an overall update on current H_4R research, emphasising that a large body of evidence identifies the H_4R as a central player in initiating and propagating immune responses, yet the variability in H_4R-mediated signals and in the pharmacological properties of H_4R ligands has to be carefully considered when characterizing H_4R functions in in vivo models of disease and translating preclinical data to clinical human settings.

Overall, this joint COST Action BM0806/EHRS conference attracted all the major players who have interest in H_4R-related research and drug development and it was characterised by intense and fruitful discussions between delegates, which are expected to meet the major challenges in understanding the functional properties and the therapeutic potential of the H_4R.

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Histamine H_4 receptor

PROGRESS AND LIMITATIONS IN H_4R RESEARCH

E. Tiligada

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

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H_4R AND NEUROINFLAMMATION: INSIGHTS FROM MOUSE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

M.B. Passani

The histaminergic system has been postulated to have a role in the pathogenesis of autoimmune diseases and there are several lines of evidence suggesting a key regulatory role of histamine in the widely used murine model, experimental autoimmune encephalomyelitis (EAE). Therefore, strategies aimed at interfering with the histamine axis may have relevance in the therapy of CNS autoimmune disease. Given the distribution of the histamine H4 receptor (H4R) on immune cells and its primary role in inflammatory functions it has become a very attractive target for the treatment of asthma and autoimmune diseases. Furthermore, recent evidence has shown the topological and functional localisation of the H4R in the CNS of both humans and rodents. However, the picture is more complex than expected, as recent data showed that the activation, and not the antagonism, of the H4R leads to reduced pro-inflammatory capacity of a subpopulation of dendritic cells found in inflamed tissues in atopic dermatitis. Results from our laboratory are also pointing in this direction, as H4R antagonists tested in EAE murine model appear to exacerbate several parameters of the disease and up-regulate H4R expression on T and dendritic cells in the spinal cord of EAE mice. Accordingly, H4R KO mice develop more severe EAE.

The complex and apparently controversial results summarized here, on the one hand provide compelling evidence that the H4R has multiple roles in the function of various cellular elements that may be associated with immune disorders. However, these observations challenge the scientific community to develop the adequate treatments for these pathological conditions.

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BEHAVIOURAL ANALYSIS OF H_4 RECEPTOR KNOCKOUT MICE

K. Rossbach, M. Bankstahl, W. Bäumer

Histamine is a well known neurotransmitter that is involved in the regulation of many different brain functions such as waking-sleep cycle, anxiety, cognition, learning and memory as well as basic homeostatic functions. All four histamine receptors ($H_{1\text{-}4}R$) are located in the brain. The expression of the H_4R in the human and rodent brain has only recently been reported and its functional role remains to be elucidated. In this study a first set of behavioural tests was performed twice with H_4R knockout mice in comparison to age-and sex-matched wild-type mice (BALB/c) (4 male and 4 female mice per group). For a general behavioural screen, a subset of tests from the Irwin screen was performed (7 males and 10 females/group). General physical characteristics or sensorimotor

reflexes showed no obvious abnormalities in the group of H_4R knockout mice. However, H_4R knockout showed an enhanced escape response to approach and struggled more when touched or restrained by tail. For analysing the general activity and anxiety-related behaviour, the open field test, the light and dark box and the elevated plus maze test were performed. Overall, the H_4R knockout mice showed only marginal differences in exploratory or anxiety-like behaviour. Furthermore, spatial learning and memory were tested in the Morris water maze test (10 females/group). The H_4R knockout showed an apparent improved performance in the Morris water maze test compared to the poor performance of the wild-type mice in this experimental setting. In summary, the H_4R knockout mice seem to react with hyperactivity to handling, but showed no gross differences in other test parameters of the Irwin screen or in their exploratory or anxiety-like behaviour. The apparent improved performance in the Morris water maze test of the H_4R knockout mice may be a first hint for a better learning and memory behaviour of these mice. Thus, the present study reveals that there are differences in the behavioural phenotype of the H_4R knockout mice, but further studies are required to confirm these differences and in more detail.

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SPECIFIC HISTAMINE H_4 RECEPTOR ANTAGONISTS ACT AS POTENT MODULATORS OF MAMMAL VESTIBULAR FUNCTION

E. Wersinger, G. Desmadryl, S. Gaboyard-Niay, A. Brugeaud, J. Dyhrfjeld-Johnsen, C. Chabbert

Histamine is a naturally occurring biological amine that exerts a range of effects on many physiological processes through the activation of four different G protein-coupled histamine receptors (H1R-H4R). Besides their localization in the immune system, H1-H4 receptors are also found in the central and peripheral nervous systems. Recent work has shown H3R expression in mammalian vestibular neurons and has shown that their antagonist, betahistine, routinely used in the treatment of vertigo can act peripherally by inhibiting the afferent discharge recorded from the vestibular nerve in several species.

We aimed to further explore the expression and role of H4R in Long Evans rat vestibular system by combining RT-PCR and immunohistochemistry experiments, as well as pharmacological and behavioral tests. Using this multidisciplinary approach we first confirmed the expression of H1 and H3 receptors and further demonstrated the localization of H4R in primary vestibular neurons cytoplasmic membrane. In vitro, whole cell patch-clamp recordings from P4 to P7 cultured vestibular neurons revealed strong and reversible inhibitory effects on evoked action potential firing by H4R antagonists JNJ10191584 and JNJ7777120 with IC_{50} in the μmolar range ($n > 5$ cells/concentration tested). In vivo, the effect of these antagonists on experimentally induced severe vestibular deficits were evaluated in three different animal models using a rating system based on multiple criteria: circling, head bobbing and head tilt, tail hanging and air righting reflexes. Each H4R antagonist significantly alleviated induced vestibular deficits by 20–30 % ($n > 8$ animals for control, sham and treated conditions). By contrast, neither of the H3 receptor antagonists tested as reference compounds had significant effects.

This study demonstrates the role of H4R antagonists in modulating vestibular function and suggests that they are strong candidates for a novel, highly efficient treatment of vertigo crisis caused by peripheral vestibular dysfunction.

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

M. Katebe, N. Lethbridge, P.L. Chazot

The histamine H_4 receptor is a G-protein coupled receptor that is primarily expressed on cells involves in inflammation and immune responses such as eosinophils, mast cells, dendritic cells and T-cells. It has been postulated to have immunomodulatory functions in these cells such as activation, migration and production of chemokines and cytokines.

Immunohistochemical studies from our laboratory and others have revealed the expression of the receptor on sensory neurons, small and medium sized diameter neurons and lamina I and II of the spinal cord suggesting a potential new target for the modulation of pain. The H_4R antagonist JNJ7777120, exhibits analgesic effects in Complete Freund's adjuvant (CFA) pain models.

The aim of the present study was to investigate the changes in H_4 receptor expression that occur in the acute (24 h) and chronic (16 days) phases of Complete Freund's adjuvant induced inflammation as compared to non-treated

animals (in collaboration with GSK (UK) and Pfizer (UK), respectively). Our hypothesis was that H_4 receptor protein would be differentially altered during the acute and chronic phases of inflammatory-induced pain.

Using our well validated anti-hH_4 receptor antibody which cross-reacts with rodent tissue, western blot analysis of skin and spinal cord tissue samples obtained from CFA-induced male Wistar rats, acute ($n = 10$) and chronic ($n = 4$) inflammatory pain models showed an apparent upregulation in the expression of H_4 receptors at the skin but not the spinal cord. In contrast, this apparent up-regulation was not observed later in the chronic phase in the skin, and an apparent down-regulation of expression was seen in the spinal cord. We are currently formally identifying the cell types where these changes are occurring, namely immune and/or neuronal. This indicates both topological and temporal differences in H_4R expression occur in inflammatory pain states

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ROLE OF HISTAMINE H_4R IN BLEOMYCIN-INDUCED PULMONARY FIBROSIS

A. Pini, D. Bani, M.B. Passani, R.L. Thurmond, H. Stark, E. Masini

Pulmonary fibrosis is a progressive and lethal illness characterized by inflammation and abnormal remodeling of lung parenchyma. No cure exists for this disease. There are various models used for the study of pulmonary fibrosis, among these, the bleomycin one is the best characterized murine model in use. Bleomycin alters oxidant/antioxidant balance and ROS overproduction activates several intracellular signaling pathways, leading to pro-inflammatory cytokine production. The histamine H_4R, expressed on cell of immune origin, plays an important role in inflammatory process. We previously demonstrated that JNJ7777120 (JNJ), a selective H_4R antagonist, potentiates the beneficial effect of naproxen in this experimental model. The aim of the present study was to investigate the role of different H_4R ligands in controlling inflammation and pulmonary fibrotic process induced by bleomycin. C57/bl6 mice were treated with vehicle, JNJ (total dose 40 mg per Kg/bw) or ST-1124 (partial inverse agonist), ST-1006 (partial agonist) and ST-994 (neutral antagonist) at equimolar doses, released by micro-osmotic pumps for 21 days. Airway resistance to inflation, an index of lung stiffness, was assayed and lung tissue processed to evaluate inflammation and fibrosis. Our results indicate that JNJ and ST-994 exert an anti-inflammatory effect, as shown by the significant decrease in the levels of PGE_2, MPO, an index of leukocyte infiltration, and TBARS, markers of oxidative stress. They also reduce the relative number of goblet cells, the thickness of smooth muscle layer (parameters of inflammation-induced adverse bronchial remodeling), the level of pro-fibrotic cytokine (TGF-β) and collagen deposition; these effects are accompanied by a decrease in airway resistance to inflation. The compounds ST-1112, partial inverse agonist and ST-1006, partial agonist, exerted a modest effect on reducing inflammation and preventing the fibrotic process.

Taken together our results indicated that H_4R blockade is associated with an anti-inflammatory and anti-fibrotic effect and may offer a new therapeutic option for the treatment of Th2-dependent lung inflammatory disease.

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

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

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THE HISTAMINE H_4 RECEPTOR BLUNTS NEUTROPHIL DEGRANULATION BY PREVENTING BETA 2 INTEGRIN-DEPENDENT SIGNALLING

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

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H_1 VERSUS H_4 ANTI-HISTAMINES IN HUMAN NEUTROPHIL OXIDATIVE BURST

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

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

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The paradigm that the histamine H_4 receptor (H4R) blockade is related to inhibition of inflammation has been

challenged by the recently reported agonist activity of JNJ7777120, the "standard" H4R antagonist, in some settings. This is in line with previous observations from our lab, which showed anti-inflammatory effects of the H4R agonists VUF8430 and VUF10460 on carrageenan-induced paw edema in the rat.

In the present study, several H4R ligands were characterized for their H4R activity (agonism, partial agonism, neutral antagonism) using the rat GTPγS binding assay and tested in vivo against carrageenan-induced paw edema in the rat.

Among the compounds tested, VUF8430 behaved as a full agonist ($\alpha = 0.92 \pm 0.02$) in the GTPγS assay and induced at 100 mg/kg subcutaneously (sc) a 92 % inhibition of paw edema; JNJ7777120, VUF10460 and clobenpropit behaved as partial agonists ($\alpha = 0.42 \pm 0.05$, 0.13 ± 0.07 and 0.21 ± 0.02, respectively) and induced 77.02, 65.58 and 66.73 % inhibition of paw edema, respectively. VUF10519 and VUF10497 were neutral antagonists in the GTPγS assay ($\alpha = 0$) and induced 38.18 and 32.36 % inhibition of paw edema, respectively, at 2 h.

In conclusion, present data suggest that the functional activity of H4R ligands in rat assays in vivo does not correlate with their functional behaviour in vitro. Surprisingly, H4R agonists were more effective than H4R antagonists as inhibitors of carrageenan-induced edema.

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

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The histamine H_4 receptor (H_4R) contributes to the regulation of several major immune functions. In antigen presenting cells, the down-regulation of pro-inflammatory cytokines and chemokines has been observed. In contrast, regarding human lymphocytes, in particular CD4 + T cells, it has been shown that the H_4R is up-regulated in Th2 polarized T cells and its activation leads to an increase in the expression of the pro-inflammatory cytokine IL-31. In human Th17 cells stimulation with either histamine or H_4R agonists enhances the production of IL-17.

In order to obtain a more comprehensive insight into the cellular activities of human lymphocytes in response to H_4R stimulation, we performed microarray-based mRNA expression profiling, using the Whole Human Genome Oligo Microarray (G4845A, ID 026652, Agilent Technologies) which contained 44495 oligonucleotide probes covering roughly 27390 human transcripts. For this purpose we treated human Th2 polarized T cells, CD8 + T cells and natural killer cells (NK cells) with the selective H_4R agonist ST-1006 and compared the mRNA expression profiles with non stimulated control cells of the same origin.

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

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

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

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Chronic spontaneous urticaria (CU) is a common skin disorder characterized by histamine (HA) release from activated skin mast cells and basophils. An underlying cause remains elusive and symptomatic therapies often lack efficacy, thus urging the need for the identification of more beneficial therapeutic strategies. Considering the recent concept of the immunomodulatory role of the HA H_4 receptor (H_4R), this study aimed to assess the relationship of blood HA levels and skin H_4R expression to the response of CU patients to standard therapy. Peripheral blood samples were collected from adult male ($n = 3$) and female ($n = 10$) CU patients of 45 ± 12 years of age, unresponsive to anti-H_1R drugs (group A) and with full remission of urticarial lesions during anti-H_1R treatment

(group B). Whole blood and serum HA levels were determined fluorometrically and presented as ng/ml of blood. H_4R expression was detected immunohistochemically on puncture biopsies (4 mm) from the urticarial lesions. Whole blood HA levels were significantly higher ($p < 0.01$) in patients with refractory CU (30.7 ± 12.3, $n = 9$) compared to responsive subjects (13.3 ± 2.3, $n = 4$). In contrast, serum HA levels were comparable in the two groups, being 7.7 ± 0.8 and 9.1 ± 1.3 for group A and B, respectively. Interestingly, a significant correlation ($r < 0.05$) between whole blood HA levels and monocyte counts was observed in group A samples. Prominent anti-$h$$H_4R$ immunoreactivity was detected in the skin biopsies, with no apparent change in total skin sample extracts. However, representative samples from the two groups showed differential anti-$h$$H_4R$ expression profiles. These data provide the first evidence linking whole blood HA levels and monocyte counts in refractory CU. The differential H_4R expression profile in skin biopsies from responsive and unresponsive patients points to a probable role of the H_4R in CU that deserves further consideration.

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

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G protein-coupled receptors (GPCRs) acquired their name from the ability to activate G proteins to induce intracellular signalling. However, not only G proteins transduce GPCR activation, also other proteins such as β-arrestins can function as signalling scaffolds. Moreover, GPCR ligands do not always activate G protein- and β-arrestin-mediated signalling to an equal amount. This phenomenon is known as *ligand-directed signalling* and has been observed for various GPCRs.

The histamine H_4R was previously shown to couple to $G\alpha_i$ proteins, but has recently been added to the list of GPCRs that display ligand-directed signalling. The well-known antagonist JNJ 7777120 was surprisingly identified as a partial agonist in a PTX-insensitive PathHunter β-arrestin recruitment assay. In addition, JNJ 7777120 induced ERK phosphorylation in a time frame typical for β-arrestin-mediated signalling.

These observations led us to re-evaluate a variety of H_4R compound classes and to investigate their ability to induce PTX-sensitive CRE activity versus PTX-insensitive β-arrestin recruitment. We have identified compounds with a strong bias towards one of the tested pathways. The newly identified biased H_4R compounds will be useful pharmacological tools to study the functional consequences of biased H_4R signalling in future research.

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

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

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

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Histamine—the biogenic monoamine—fulfills important physiological functions through four histamine receptor subtypes (H_1-H_4). The youngest member of this receptor family is the H_4 receptor (H_4R) which was discovered at the turn of 2000 and 2001 year independently by several research groups. Because of H_4R expression mainly in cells and tissues of immune system (monocytes, mast cells, dendritic cells, eosinophils, basophils, T-leukocytes) its

role in inflammatory and (auto)immunological processes and disorders was suggested. Positive effects were observed in treatment of animal models of some diseases (e.g. allergic rhinitis, asthma, pruritus or pain) in the presence of H_4R antagonists/inverse agonists.

Extending our SAR investigation in the group of 1,3,5-triazine derivatives—based on previous results and literature data we searched for new potent and selective H_4R ligands in the group of (4-methylpiperazin-1-yl)-1,3,5-triazin-2-amines by changing aryl substituent in 6-position to heteroaryl ring system, e.g. (un)substituted thiophene, benzothiophene, pyridine or imidazopyridine.

The compounds were obtained by the direct reaction of appropriate carboxylic esters with guanidine derivative. In silico predictions of toxicity and drug-likeness by newly obtained compounds were also carried out. Compounds were evaluated for their affinity at H_4R with radioligand binding assays on transiently expressing recombinant human H_4R in the *Sf9* insect cells using [^{3}H] histamine as radioligand.

As one of the most promising substitution pattern the thienyl derivatives were characterized with significant affinity to H_4 receptor, e.g. 4-(3-chlorothienyl-2-yl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine showed a K_i value of 137 nM.

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MICROSATELLITE REPEAT EXPANSIONS IN HUMAN HISTAMINE$_4$ RECEPTOR GENE

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Microsatellite repeat expansions have been implicated in a number of neurological disorders in humans, e.g., Fragile X syndrome, Huntington's disease and Kennedy's disease. Both loss-of-function and gain-of-function mechanisms are recognised from expanded trinucleotide repeats, and such microsatellites can be located in both coding and non-coding regions of the gene. This study targets genetic polymorphism in the promoter region of the human histamine$_4$ receptor (hH_4R), which contains variable number of tandem repeat polymorphisms (VNTR). This VNTR is a CAA triplet repeat expansion and ranges from 10 to 19 repeats (10R and 19R, respectively). In preliminary unpublished data it was indicated that the trinucleotide repeats 10R, 13R, 14R and 15R, are more common in schizophrenia patients. The aim of this study was to investigate the association of this genetic variation to schizophrenia and to assess promoter activity resulting from this polymorphism. Linkage disequilibrium (LD) analysis was carried out on HapMap samples between the VNTR in the promoter region and known Single Nucleotide Polymorphisms (SNPs) at this locus. The SNPs at hH_4R were tested for association with schizophrenia as part of genome wide association studies (GWAS) conducted by the International Schizophrenia Consortium (ISC). Genotyping showed that the 13R allele is in high LD with rs17797945, while the 10 microsatellite is in high LD with rs628764 and rs615283. The SNP rs17797945 is weakly associated with schizophrenia in the ISC GWAS (P 0.007876). The promoter region including the variations in VNTR have been cloned and are currently being subcloned in a pGL3 promoter-less vector for assessment of promoter activity. This assay will indicate the functional impact which this length polymorphism has on gene expression.

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SILENCING HUMAN H_4R GENE BY NOVEL TRIPLEX-FORMING MOLECULE

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

prevents biofeedback loops from becoming effective drug resistance mechanisms.

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

The H_4R-TFM developed bound efficiently with its DNA target at physiological temperature and pH, prevented amplification in RT-PCR and, therefore, can now be potentially used to disrupt in vitro transcription and prevent replication of DNA containing a binding site for the hH_4R-TFM.

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

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Histamine is known to be synthesized in the glomeruli, to increase second messenger levels in isolated glomeruli and to influence renal hemodynamics, including microcirculation. Previous studies demonstrated the glomerular expression of histamine H_1 receptors (H_1R) and H_2R, while the most recently discovered H_3R and H_4R were poorly investigated. The aim of this study was to evaluate the H_4R expression in the rat kidney. As renal histamine concentration was found to be significantly increased in streptozotocin (STZ)-treated rats, a further aim was to evaluate whether the kidney H_4R density varies in diabetic rats. Insulinopenic diabetes was induced in 12 out of 24 8-week-old male Wistar rats by a single i.v. injection of STZ, and animals were sacrificed 6 weeks later. Serum and urinary evaluation was performed to assess glycaemia, creatinine clearance and proteinuria. The kidneys were collected and processed for PCR or immunohistochemistry analyses. Diabetic rats (glycaemia >300 mg/dl) had a higher creatinine clearance and proteinuria than nondiabetic animals. The H_4R mRNA expression in control rats was at a very low level. Consistently, H_4R was undetectable by immunohistochemistry using a well validated anti-H_4R antibody. In contrast, in diabetic rats H_4R was profoundly *upregulated*.

Immunohistochemistry experiments revealed a higher immune-positivity in the cortex and medulla in comparison to the papilla. To ascertain the cellular localization of H_4R, colocalization experiments were performed. When H_4R immunostaining was compared to that of Tamm-Horsfall glycoprotein, a close overlap in expression topology was observed.

In conclusion, our results indicated that H_4R is expressed in the kidney of healthy rats, although at a very low level, and it is upregulated in diabetic male rats. This receptor is expressed by resident renal cells of the thick ascending limb of the loop of Henlé, thus suggesting a possible role in modulating the transmembrane soluble transport processes.

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

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We have previously reported the presence of the histamine H_4 receptor (H_4R) in benign and malignant lesions and cell lines derived from the human mammary gland. The H_4R is one of the main subtypes responsible for the histamine-induced responses in MDA-MB-231 breast cancer cells. The aim of the present work was to evaluate the effects of H_4R ligands on the survival, tumour growth rate, metastatic capacity and molecular pattern of expression of antigens related with the proliferative and apoptotic potential in a breast cancer experimental model. For that purpose, we established xenografted tumours of the highly invasive human breast cancer cell line MDA-MB-231 in immune deficient nude mice. We employed the following H_4R agonists: histamine (1 and 5 mg/Kg, sc), clozapine (1 mg/Kg, sc), and the experimental compound JNJ28610244 (10 mg/Kg, sc).

Results indicate that developed tumours were highly undifferentiated and that the H_4R was the major histamine receptor expressed. They also exhibited high levels of histidine decarboxylase, histamine content and proliferation marker (PCNA) while displaying low levels of apoptosis. Mice of the untreated group displayed a median survival of 60 days, and a tumour doubling time exponential growth of 7.4 ± 0.6 days. A significant

decrease in tumour growth evidenced by an augmentation of the tumour doubling time was observed in H_4R agonist groups (13.1 ± 1.2, $P < 0.01$ in histamine group; 15.1 ± 1.1, $P < 0.001$ in clozapine group; 10.8 ± 0.7, $P < 0.01$ in JNJ28610244 group). The effect was associated with a decrease in the expression levels of the H_4R (32.8 vs. 6.6 % and 3.0 in clozapine and JNJ28610244 groups, respectively, $P < 0.05$) and of the PCNA (83.1 vs. 63.2 %, 64.2, 70.5 % in histamine, clozapine and JNJ28610244 groups, respectively, $P < 0.05$). Histamine treatment significantly increased median survival (78 days; ***$P < 0.0005$, Log rank test and Gehan-Breslow-Wilcoxon test). We conclude that histamine through the H_4R exhibits a crucial role in tumour progression. Therefore, H_4R ligands offer novel therapeutic potential as adjuvants for breast cancer treatment.

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ESTABLISHMENT OF A HISTAMINE METHODS AND TOOLS DATABASE

H.G. Schwelberger

COST Action BM0806 Recent Advances in Histamine Receptor H_4R Research brings together scientist and industrial partners from many different disciplines, who provide enormous expertise in their respective field. In order to make this expertise transparent and usable for all action members and for histamine researchers in general, one goal of Working Group 1 is to establish and run an internet database covering all methods and tools available at participating institutions.

In the first phase to build this database, a simple questionnaire was used to collect, evaluate and combine information on available methods and tools from individual participants. This methods and tools overview will serve as a contents and reference point for users. In the second phase, detailed protocols and descriptions of tools are collected from individual contributors and made available for users in a common, easy to use format. In the third phase, the database will be constantly updated with new information and errors will be corrected based on user feedback.

In its final version, this histamine methods and tools database should provide a comprehensive collection of all available methods (techniques, assay systems, cell systems, animal models, patient based studies), include ready-to-use protocols, give contact information for method based inquiries (technical help), inform about source of critical tools (antibodies, reagents, compounds), and finally identify areas where new methods, techniques or tools should be developed.

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Neurological aspects of histamine

UNDERSTANDING HOW HISTAMINE INDUCES NEURON DIFFERENTIATION

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Histamine is a neurotransmitter in the central nervous system. This amine is one of the first neurotransmitters to appear during development and it reaches its maximum concentration at the same time as neuron differentiation peak. This suggests that histamine has an important role in neurogenesis. We have previously shown that histamine is able to increase neuronal differentiation of neural stem cells in vitro, by activating the histamine H_1 receptor. In this study, we start to explore the mechanism involved in neuronal differentiation stimulated by histamine. We used rat neural stem/progenitor cells from embryonic day 14 in passage two. We performed two protocols of histamine treatments; one when it was present during both cell proliferation and differentiation and the other where histamine was only present during cell differentiation.

Immunocytochemistries were performed to evaluate cell division and neuron differentiation. RT-PCR or qRT-PCR were used to explore the expression of some important transcriptional factors involved on cell commitment. Results indicate that histamine is required during cell proliferation in order to increase neuron differentiation, probably by increasing neuron commitment, since histamine is able to increase asymmetric cell division during the proliferative phase as well as the expression of *FGF-receptor type 1*, *Neurogenina 1* and *Prospero 1* expression. We proposed that the increase on deep layer cortical neurones, measured by an increase on FOXP2-positive cells, due to histamine is related to: (a) an increase on asymmetric cell division and (b) an increase on *FGF-receptor 1*, *Prospero 1* and *Neurogenin 1* expression. These effects might be related to the activation of histamine H_1 receptor, since the presence of chlorpheniramine prevented the increase on FGF-receptor 1 expression.

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

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The histamine H_3 receptor (H_3R) is mainly located on nerve terminals and controls release of histamine and other transmitters, including dopamine. The alternative splicing of the human H_3R gene produces several receptor isoforms, of which variants of 365 and 445 amino acids (hH_3R_{365} and hH_3R_{445}) are expressed in brain. The aim of this work was to determine, by using a cellular model, whether these two isoforms regulate in a different manner neurotransmitter release.

Two sub-lines of human neuroblastoma SH-SY5Y cells stably expressing either isoform (hH_3R_{365} or hH_3R_{445}) were generated with expression levels of 583 ± 151 and 805 ± 175 fmol/mg protein and Kd values for [^{3}H]-N-methyl-histamine of 0.90 ± 0.13 and 0.86 ± 0.12 nM, respectively. There were no significant differences in the affinities of both isoforms for the H_3R ligands histamine (pKi values 8.50 ± 0.10 and 8.55 ± 0.07), R-α-methyl-histamine (RAMH, 9.45 ± 0.06 and 9.63 ± 0.06) or clobenpropit (8.70 ± 0.09 and 8.95 ± 0.12).

The H_3R agonist RAMH was more efficacious to inhibit forskolin-induced cAMP accumulation in the SH-SY5Y-hH_3R_{445} cell line (-70.6 ± 5.2 % vs. -38.4 ± 5.4 % for the SH-SY5Y-hH_3R_{365} cells), without significant difference in the pIC50 estimates (8.77 ± 0.17 vs. 8.42 ± 0.14).

Neurotransmitter release was assayed in cells differentiated by treatment for 7 days with 12-O-tetradecanoyl-phorbol-13-acetate, TPA (20 nM), and retinoic acid (10 μM). The release of [^{3}H]-dopamine induced by depolarization with high K^+ (100 mM) was significantly reduced by RAMH (100 nM), but the effect was significantly larger in the SH-SY5Y-hH_3R_{445} cell line (-30.9 ± 4.2 %) than in the cells expressing the hH_3R_{365} isoform (-15.1 ± 4.8 %).

Our results indicate that in accord with previous data for other signaling pathways, the hH_3R_{445} isoform is more efficacious in inhibiting depolarization-evoked neurotransmitter release.

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DYNAMIC REGULATION OF HISTAMINERGIC AND DOPAMINERGIC NETWORKS IN ZEBRAFISH BRAIN

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

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HUMAN ASTROCYTES TRANSPORT HISTAMINE THROUGH PLASMA MEMBRANE MONOAMINE TRANSPORTER

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

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

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

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DISCOVERY OF NOVEL H_1R AND H_3R MODULATORS THROUGH A MULTIPLEXED SCREENING STRATEGY UTILIZING SMALL MOLECULES FROM A CNS DOS FOCUSED LIBRARY

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In an effort to synthesize a diverse library of unique compounds biased for CNS drug like properties we utilized

in silico, in vitro, cellular and in vivo models to inform library design. An azetidine based core provided a versatile common intermediate en route to a variety of fused, bridged and sprirocyclic ring systems. A phenylethylamine structural motif embedded within the core scaffold provides a pharmacophoric element common to a number of hormones, neurotransmitters, drugs and natural products known to affect the central nervous system. Utilizing an unbiased and multiplexed screening strategy on representative scaffolds we discovered novel, relatively potent and highly selective compounds which modulate the H_1 and H_3 receptors in vitro and in vivo. These compounds act as H_3 antagonists/inverse agonists and functionally selective H_1 agonists and display antidepressant and cognitive enhancing effects in mice. Recent genetic findings implicating histaminergic pathways in the etiology of Tourette syndrome and possible overlap with autism, OCD and ADHD offer intriguing opportunities for therapeutic intervention.

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HISTAMINE PROMOTES NEURONAL DIFFERENTIATION OF CULTURED MIDBRAIN NEURAL PRECURSOR CELLS BUT REDUCES DOPAMINE NEURONAL NUMBERS IN VITRO AND IN VIVO

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

During embryonic development, histamine (HA) is one of the first neurotransmitters to appear in the central nervous system (CNS). Particularly, in the region of rat ventral midbrain (VM), HA increases its concentration fivefold at embryonic day 14–16 and then it decreases until birth, reaching the low concentrations that are present in the adult brain. Neuronal differentiation in the VM correlates temporally with the increase in the concentration of HA in this region. The role of HA during brain development is unknown. In this work, we studied the correlation between HA increases with the process of neurogenesis on rat VM in vitro and in vivo. The effect of increasing HA concentrations (from 100 nM to 1 mM) during proliferation, differentiation and cell death of VM neural precursor cells (NPC) in vitro were analyzed. We found that VM cultures express HA receptors, and that 10 μM HA significantly increased neuronal differentiation from 21 to 37 %. Pharmacological assays showed that the effect of HA on neuronal differentiation is mainly due to activation of H_2 receptors. We found that almost every HA concentration used increased twofold apoptotic cell death compared to control condition, evaluated by TUNEL assay. Also the proportion of dopaminergic neurons was significantly decreased after 1 mM HA treatment of cultured VM NPC. All experiments were performed by triplicate. We also evaluated the role of HA in vivo through intrauterine injections on the developing brain of Wistar pregnant rats. We found that HA administration decreased the number of dopaminergic neurons in the VM. This is consistent with the in vitro data. Results are representative of a cohort of 5 experimental animals. Thus, these results imply that HA might be acting as a transient developmental signal that modulates neurogenesis and dopamine neuron differentiation/survival in the rat midbrain.

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THE HISTAMINE H_3 RECEPTOR ANTAGONIST A-960656 IS EFFECTIVE IN ANIMAL MODELS OF OSTEOARTHRITIS AND NEUROPATHIC PAIN

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Histamine H_3 receptor antagonists have been widely reported to improve performance in preclinical models of cognition and efficacy in pain models has recently been described. A-960656 was profiled as a new structural chemotype. A-960656 was potent in vitro in histamine H_3 receptor binding assays (rat $K_i = 76$ nM, human $K_i = 21$ nM), exhibited functional antagonism in blocking agonist-induced [^{35}S]GTPγS binding (rat H_3 $K_b = 107$ nM, human H_3 $K_b = 22$ nM), and was highly specific for H_3 receptors in broad screens for non-H_3 sites. In a spinal nerve ligation model of neuropathic pain, oral doses of 1 and 3 mg/kg were effective 1 h post dosing with an ED_{50} of 2.1 mg/kg and a blood EC_{50} of 639 ng/ml. In a model of osteoarthritis pain, oral doses of 0.1, 0.3, and 1 mg/kg were effective 1 h post dosing with an ED_{50} of 0.52 mg/kg and a blood EC_{50} of 233 ng/ml. The antinociceptive effect of A-960656 in both pain models was maintained after sub-chronic dosing up to 12 days. All studies used male Sprague–Dawley rats weighing 250–300 g at the time of testing. A-960656 had excellent rat pharmacokinetics ($t_{1/2} = 1.9$ h, 84 % oral bioavailability) with rapid and efficient brain penetration, and was well tolerated in CNS behavioural safety screens. A-960656 has properties well suited to probe the pharmacology of histamine H_3

receptors in pain. Its potency and efficacy in animal pain models provides support to the notion that histamine H_3 receptor antagonists can effectively attenuate nociceptive processes.

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THE HISTAMINE H_4 RECEPTOR IS EXPRESSED ON BOTH PEPTIDERGIC C- and Aδ SENSORY FIBRES AT THE LEVEL OF THE RAT SKIN, DORSAL ROOT GANGLION AND DORSAL HORN OF THE SPINAL CORD

M. Katebe, P.L. Chazot

The histamine H_4 receptor (H_4R) is the most recent receptor identified belonging to the histamine receptor subfamily of G-protein coupled receptors (GPCR). Until recently the H_4 receptor was thought to be largely expressed on haematopoietic cells and as a result, is a possible key new target for inflammatory disorders.

Immunohistochemical studies from our laboratory and others have revealed strong H_4R expression on the soma of cervical and lumbar dorsal horn sensory neurons with more intense staining on small and medium diameter cells, and subpopulations of dorsal root ganglia (DRG), suggesting a potential role in nociception and a new target for the modulation of chronic inflammatory pain disorders. The H_4R ligand, JNJ7777120, exhibits profound antipruritic and analgesic effects in the Freund's Complete Adjuvant (FCA) chronic pain model, the latter where spinal NMDARs are hyperactivated (unpublished data). Our hypothesis was that the H_4R would be expressed on C-fibres.

We have used a double immunofluoresence labelling approach to determine the possible co-localisation of H_4Rs on peptidergic C-(Substance P) and Aδ (CGRP) nociceptive fibres at the level of skin, DRG and the dorsal horn neurones of the spinal cord in the rat. At all levels of the sensory pain pathway tested, our results surprisingly show expression on both peptidergic C-fibres and on Aδ fibres, the latter particularly prominent at the level of the DRG and the skin. This expression profile contrasts with the H_3R, which was found to be expressed only on a subset of Aδ fibres, and is undetectable on C-fibres.

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

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

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

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Histamine (HA) is a key regulator of experimental allergic encephalomyelitis (EAE), the autoimmune disease

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

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

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

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WAKING ACTION OF URSODEOXYCHOLIC ACID INVOLVES HISTAMINE AND $GABA_A$ RECEPTOR BLOCK

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Since ancient times ursodeoxycholic acid (UDCA), a constituent of bile, is used against gallstone formation and cholestasis. We show now that UDCA (35 mg/kg) promotes wakefulness during the active time of the day, lacking this activity in histamine-deficient mice. In cultured hypothalamic neurons UDCA synchronized the firing, an effect not present under the $GABA_AR$ antagonist gabazine (10 μM). In histaminergic neurons recorded in rat slices UDCA reduced amplitude and duration of spontaneous and evoked inhibitory postsynaptic currents (IPSCs) but left the firing unchanged. In acutely isolated mouse histaminergic neurons UDCA blocked GABA-evoked currents and sIPSCs starting at 10 μM (IC_{50} = 70 μM) and did not affect NMDA- and AMPA-receptor mediated currents at 100 μM. The potency of UDCA inhibition of $GABA_A$-receptors was not different between rat and mouse. Recombinant $GABA_A$ receptors composed of α1, β1-3 and γ2L subunits expressed in HEK293 cells displayed similar sensitivity to UDCA as native $GABA_A$ receptors. The mutation α1V256S known to reduce the inhibitory action of pregnenolone sulphate reduced the potency of UDCA. The mutation α1Q241L, which abolishes $GABA_AR$ potentiation by several neurosteroids had no effect on $GABA_AR$ inhibition by UDCA. In conclusion, UDCA can enhance alertness through disinhibition of the histaminergic system.

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

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

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THE ANOREXIANT EFFECT OF OLEOYLETHANOLAMIDE IS MODULATED BY NEURONAL HISTAMINE

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The modulatory effect of both brain histamine (HA) and Oleoylethanolamide (OEA) on feeding behaviour is robust, but nothing is known about the temporal or causal relationship between HA and OEA in controlling food intake. To learn if OEA affects feeding behaviour via the histaminergic system we measured food consumption in normal and genetically or pharmacologically HA-deprived mice. We use sv129 WT and HDC-KO and CD1 male mice that were food deprived for 12 h and then received pharmacological treatments. Food consumption was measured every 15 min for the 1st h and at increasing intervals for the following 24 h. OEA-treated (10 mg/kg i.p. n = 16) WT mice ate significantly less than vehicle-treated WT mice (n = 16). However, the anorexiant effect of OEA was attenuated in HDC-KO mice (n = 28). OEA anorexiant effect was also attenuated in HA depleted CD1 (5 μg α-fluoromethyl-histidine i.c.v. n = 20), but not in control mice (n = 20). Treatment with the H_3R antagonist ABT239 (3 mg/Kg i.p.) potentiated the effect of OEA (5 mg/kg, i.p. n = 20/treatment). In order to investigate if changes in neuronal activation are related with the behavioral differences, we evaluated the c-Fos expression in HDC-KO and WT (n = 3–5/treatment) mice using immunohistochemistry in brain areas involved in feeding behavior: Infralimbic Cortex (IL-Cx), Nucleus Accumbens (Nac) and Paraventricular Nucleus (PVN). Mice fasted for 12 h were treated with OEA (10 mg/kg i.p.) or vehicle and sacrificed after 2 hs. OEA treatment decreased the number of c-Fos$^+$ cell nuclei in the Nac of both HDC-KO and WT mice. No changes were observed in the IL-Cx of either genotype. However, in the PVN an increase of c-Fos expression was observed in OEA-treated WT but not in HDC-KO mice. Our results indicate that the histaminergic system is involved in the anorexiant effects of OEA and that disregulation of PVN neuronal activity may be responsible for the partial lack of OEA efficacy in HA deficient mice.

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

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

ST-1283 was investigated both in vitro and in vivo. Oral ethanol, saccharin and quinine intake was assessed in a two-bottle choice paradigm using escalating concentrations of alcohol or tasting solutions. In these screenings, 2.5, 5, 10 and 20 % alcohol, 0.04 % and 0.08 % saccharin, and 0.02 mM and 0.04 mM quinine solutions were used.

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

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SEXUAL AROUSAL, A ROLE OF HISTAMINE AND OREXINS?

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Sexual arousal ensures the necessary waking state allowing anticipation and performance of sexual activities and so is a prerequisite for reproduction. We hypothesize that a full expression of such a behavioural state requires highly optimal convergent and divergent activities of the brain arousal systems, notably histamine (HA) and orexins (Ox). To test our hypothesis, wild type (WT) and histidine-decarboxylase (Hdc) or OxKO mice were chronically implanted for EEG and sleep-wake monitoring under baseline conditions (12 h light/dark cycle) and during the sexual arousal test. This consisted of introducing a female mouse for 4 h into the habitual cage of a male mouse; the two mice were separated by a transparent plexiglass with a maximal number of holes (diameter = 14–16 mm), allowing some physical contact, but preventing copulation. Placement of a male mouse was used as control. The test was performed during both light and dark phases. Firstly we found that the presence of a female mouse elicited in the male a significant increase in waking (+49 % vs. baseline lightness, $n = 32$, $p < 0.001$). This increase is not significant if another male was introduced (+10 % vs. baseline, $n = 32$, $p = 0.2$). This sexual arousal appeared to depend on sex hormones as ovariectomized female mice or those pretreated with Tamoxifen (an estrogen receptor antagonist) did not elicit significant sexual arousal in the male mice. Also male mice, pretreated with Flutamide (an androgen receptor antagonist), did not show any additional waking in the presence of a female. Additionally, acute application of α-FMH (a specific inhibitor of HA synthesis) or SB-334867 (Ox1-receptor antagonist) both markedly impaired the sexual arousal in the male mice. Finally, the sexual arousal appeared intact in Hdc KO mice, whilst Ox KO mice showed signs of impairment. This all indicates that both the HA and Ox systems, probably driven by sex hormones, participate in the promotion of sexual arousal. Chronic HA loss may be compensated by up-regulated compensatory mechanisms involving the Ox system.

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Immunological aspects of histamine

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

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

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HISTAMINE CONTRIBUTES TO AN INCREASED RANKL/OPG RATIO THROUGH ALTERED NR4A ACTIVITY IN HUMAN CHONDROCYTE CELLS

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Histamine promotes immune complex-induced vascular leakage, a critical event leading to joint specific autoimmune damage in vivo. Increased histamine levels in synovial fluid, tissue and cartilage of rheumatoid- (RA) and osteo- (OA) arthritis patients support a role for histamine in regulating cartilage homeostasis. We aim to elucidate the histamine receptor-mediated signaling pathways, transcriptional events and target gene expression in human chondrocyte cells.

Histamine modulation of cartilage destruction was assessed histologically by Safranin-O staining and proteoglycan release. Histamine receptor (HR1,2,3,4) dependent regulation of nuclear transcription factors NR4A1-3; receptor activator of NF-κB-ligand (RANKL); and osteoprotegerin (OPG) mRNA levels were measured in human primary ($n = 8$) and SW-1353 chondrocyte cells using QPCR and selective HR antagonists. sRANKL and OPG protein levels were determined by ELISA. NR4A protein levels and trans-activity were evaluated by western, immunocytochemistry and luciferase reporter assays. Stable depletion of NR4A1-3 was achieved by lentiviral transduction of NR4A shRNA.

Primary human chondrocytes express differential steady state levels of HR1-4 mRNA. In combination with TNFα, histamine significantly promotes cartilage proteoglycan depletion. Histamine selectively signals through HR1 and HR2 to modulate RANKL and NR4A2 expression, with modulation significantly reduced in cells pre-treated with inhibitors of PKA, MAPK and NF-κB signalling pathways. Histamine robustly modulates RANKL, with modest effects on OPG, leading to significantly increased RANKL/OPG mRNA and protein ratios. Stable knockdown of NR4A1-3 expression results in reduced endogenous OPG levels and the loss of histamine-dependent regulation of RANKL expression.

Histamine, via HR1 and HR2, may contribute to the development of inflammatory joint disease by enhancing the expression ratio of RANKL/OPG through altered NR4A activity in human chondrocyte cells.

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HISTAMINE DOWNREGULATES SKIN BARRIER PROTEINS: A POTENTIAL ROLE IN THE PATHOGENESIS OF ATOPIC DERMATITIS

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Patients with atopic dermatitis have defects in epidermal keratinocyte differentiation and skin barrier function. These defects result either from inherent alterations in the expression of differentiation-associated proteins and/or the influence of inflammatory mediators on terminal keratinocyte differentiation. Skin mast cells participate in skin inflammatory reactions by the release of various soluble mediators, most prominently histamine. Here we studied the effect of histamine on epidermal keratinocyte differentiation in monolayer cultures, in an in vitro organotypic skin model and in human skin explant cultures. The expression of differentiation-associated proteins was assessed by quantitative realtime-PCR, Western blot and

immunofluorescence labeling. Histamine reduced the expression of filaggrin, loricrin, keratin 1 and keratin 10 by up to 90 % in keratinocyte monolayer culture and in human in vitro skin models. The influence of histamine on the expression of differentiation associated proteins was dose-dependent and only detectable when histamine was added to the cultures before the onset of keratinocyte differentiation. Accordingly, we did not find a change in the expression of differentiation-associated proteins in intact human skin biopsies after ex vivo treatment with histamine. Furthermore, addition of histamine to organotypic skin cultures reduced the expression of the cell–cell contact proteins corneodesmosin and occludin and perturbed the inside-out barrier function of the epidermis. Taken together these findings suggest an important role of histamine as deregulator of epidermal keratinocyte differentiation, contributing to the sustained skin barrier defects observed in atopic dermatitis.

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LATE SIGNALING EVENTS FOR HISTAMINE RELEASE BY MAST CELLS

U. Blank

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

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EICOSANOIDS: PERFORMING LIPIDOMICS ON ACTIVATED MAST CELLS

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

variety of oxylipins and also a difference in the ability between mast cells with different phenotypes.

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THE ALLERGIC EFFECTOR UNIT: MAST CELLS—EOSINOPHILS INTERACTIONS REGULATE THE ALLERGIC RESPONSE

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Mast cells and eosinophils, the key effector cells in allergy, co-localize particularly in the late and chronic stages of allergic inflammation. Recent evidence from our laboratory has outlined a specialized "allergic effector unit" (AEU) in which mast cells and eosinophils communicate via both soluble mediators and physical contact. Our objective was to evaluate the functional impact of this bi-directional crosstalk on the cells' survival and effector activities.

Human and murine mast cells and eosinophils were co-cultured under various conditions for a few hours or 1–3 days, and in selected experiments cell–cell contact was blocked. Cell survival, migration and mediator release were examined. Moreover flow cytometry was used to stain intracellular signaling molecules and surface receptors.

Mast cells significantly enhanced eosinophil survival both by soluble mediators and physical contact involving CD48-2B4 binding. Furthermore resting and IgE-stimulated mast cells led to eosinophil migration and activation through a paracrine-dependent mechanism. Eosinophils were found to enhance basal mast cell mediator release and IgE-mediated mast cell degranulation through CD48-2B4 interactions. Increased phosphorylation of activation-associated signaling molecules and enhanced release of tumor necrosis factor (TNF)-α were observed in long-term co-cultures. Eosinophils also showed enhanced expression of intercellular adhesion molecule (ICAM)-1, which depended on direct contact with mast cells.

In conclusion, our findings describe a new role for mast cell/eosinophil interplay in augmenting short and long-term function in both cells, in a combined physical/paracrine manner. This enhanced functional activity may thus critically contribute to the perpetuation of the inflammatory response in allergic conditions.

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

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

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

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

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

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

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

HLMC were generated by physical and enzymatic disruption of lung tissue. Cells were incubated with or without antagonist (50 min), then agonist (10 min) before challenge with anti-IgE (25 min). Histamine released into the supernatants was assayed using an automated fluorometric technique. To determine cyclic-AMP levels, purified HLMC were incubated (10 min) with agonist, the reaction terminated using ice-cold ethanol and cyclic-AMP measured using commercially available EIA kits.

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

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

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

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

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

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

Mast cells are densely granulated cells traditionally associated with the pathogenesis of allergic reactions. However, their additional roles are increasingly being recognised. As examples, mast cells are involved in cell-mediated immune reactions, are a component of the host reaction to infection, and have functions in tumour angiogenesis and tissue repair. The aim of this work was to conduct an assessment of the potential anti-allergic activity of a novel series of cyclic amino derivatives of tetralone and benzosuberone. The primary steps in their synthesis

involved a Wohl-Ziegler bromination step followed by a substitution reaction with the appropriate amine. Alkylation of the secondary amines thus formed was effected with a series of alkyl halides to afford the title compounds for evaluation. Percoll-purified rat peritoneal mast cells were used for the in vitro studies which employed compound 48/80 and calcium ionophore A231867 to induce degranulation. The in vivo passive cutaneous anaphylaxis assay was used to evaluate the most active compounds from the in vitro work. In vitro investigation of the mast cell stabilising activity revealed that optimal activity appeared to reside in a tertiary amine bearing either the parent bicyclic system, an unsaturated cyclohexene, or a benzyl or substituted benzyl motif. It further appears that the unsaturated alicyclic system on nitrogen is critical for in vivo activity. This suggests that, while ring expansion of the hydroaromatic core is permissible without loss of activity in vivo, there must be an unsaturated alicyclic component.

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

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More than 15 % of people in the western population suffer from allergy or asthma. In order to develop new and more effective therapies, a better understanding of the molecular mechanisms underpinning this chronic disease is required. Histamine, released by activated mast cells and immune cells, causes many of the symptoms associated with allergy and asthma. Histamine is recognized by 4 different histamine receptors (H1R-H4R), each inducing its own signaling cascade. H2R is known as an immune regulatory receptor but its role in allergy is not well described. We used a murine model of allergic airway inflammation similar to asthma to investigate the role of H2R in this disease. For this female H2R-/- mice and BALB/c wt mice were sensitized i.p. and OVA-aerosol challenged. Mice lacking H2R showed significant increased cell numbers in bronchoalveolar lavages, mainly due to elevated eosinophil numbers. Lung histology confirmed increased inflammatory scores in knockout animals. Furthermore, in vitro re-stimulation with OVA induced higher Th1 and Th2 cytokine release from single cell suspensions from lungs, spleen and lymphnodes. Taken together, H2R-/- mice develop more severe allergic airway inflammation. CD4 + CD25 + Foxp3 + Treg numbers were evaluated by flow cytometry in Peyer's patch, mesenteric lymph nodes, spleen, lung-draining lymph nodes, lung tissue and bronchoalveolar lavages. Treg numbers were similar in wildtype and knockout animals at all sites, with a tendency towards increased Treg numbers in H2R−/− inflamed lung, perhaps related to altered anti-inflammatory compensatory mechanisms in the H2R−/− animals.

In conclusion, H2R is an important immunoregulatory receptor that influences the severity of allergic airway inflammation in murine models. In addition, the increased severity of disease was not associated with decreased Treg numbers suggesting that other immune cell populations may be directly influenced by histamine signaling through the H2R.

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DIFFERENTIAL ROLE OF HYPOXIA-INDUCIBLE FACTOR-1 IN HUMAN MAST CELL AND BASOPHIL RESPONSES

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

mast cells was not controlled by HIF-1. We conclude that HIF-1 accumulation plays a crucial role in sustaining human allergic effector cell survival and function. This transcription complex facilitates the generation of both pro-angiogenic and inflammatory cytokines in mast cells but has a differential role in basophil stimulation comparing innate immune stimuli with IgE-dependent triggering.

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

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Histamine plays an important role in immune system disorders and allergies. Since studies about the relation of the newly discovered histamine H4 receptor with functional properties of phagocytes and phagocyte-derived reactive oxygen species (ROS) generation are very rare, we focused on evaluating histamine effects on functional activity of human leukocytes and on antioxidant properties of histamine.

The ability of isolated leukocytes or leukocytes in the whole blood of healthy human volunteers to produce ROS after histamine or the H4 receptor agonist 4-methylhistamine treatment (10^{-8}–10^{-4} M) was tested by luminol-enhanced chemiluminescence (CL). Antioxidant properties of both compounds were measured by CL (TRAP) and fluorescence (ORAC) analyses.

According to their basic level of spontaneous ROS production in whole blood and their responsiveness to the histamine treatment, the healthy volunteers could be divided into three groups. Group 3 is characterised by higher level of ROS production. In this case, both histamine and 4-methylhistamine dose-dependently inhibited the CL response of phagocytes. While there was no significant effect on the ROS production by either of the compounds in Group 2, both compounds in the lowest concentrations tested significantly increased ROS production in Group 1. Then the ROS production gradually decreased with increasing concentrations of tested compounds reaching the level of the control when the highest concentration of histamine or 4-methylhistamine was used. The differences among groups and the effects of histamine and 4-methylhistamine were less profound in isolated leukocytes. Neither histamine nor 4-methylhistamine had any antioxidant potential against reactive oxygen species.

It can be concluded that histamine modulates the activity of cells of the immune system, especially of phagocytes, in a concentration dependent manner. H4 receptors could at least partially play a role in this phenomenon.

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

F. Jantzen, T. Werfel, R. Gutzmer

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

Monocytes and myeloid dendritic cells (mDC) were isolated from human PBMC. After pre-incubation with different histamine concentrations (10^{-5}–10^{-10} mol/l) or 4-methylhistamine (10^{-5} mol/l) the cells were stimulated with PolyI:C for different time periods (2–24 h). Supernatants were analysed by protein array for the expression of several cytokines. The concentration of IP-10 in the supernatants was quantified by ELISA. To analyse which is the responsible histamine receptor we pretreated the cells with different receptor antagonists for 30 min (H_2R: ranitidine, H_4R: JNJ7777120).

A pre-stimulation with histamine caused a significant decrease of PolyI:C-induced expression of CXCL10 in a time- and dose-dependent manner in monocytes and myeloid dendritic cells. We observed this effect in protein arrays and ELISA with cell supernatants. Additional blocking with specific H_2R and H_4R antagonists showed that the observed effect is solely mediated via H_2R in monocytes, but appears to be mediated also via H_4R in mDC.

Taken together our results show that the Th1-associated chemokine CXCL10 is downregulated by histamine in monocytes and mDC. There appear to be differences in the histamine receptor responsible for this effect between monocytes and mDC, which is currently under investigation. This represents a new mechanism how histamine fosters a Th2 milieu by downregulating Th1 cytokines.

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

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The novel concept of the immunomodulatory role of histamine (HA) is supported by the primary distribution of the recently identified HA H_4 receptor (H_4R) in immune cells. This study aimed to investigate the effects of 2,4-diaminopyrimidine derivatives with different efficacies on the putative H_4R-mediated automodulatory HA function in the normal and inflamed rat conjunctiva. Male Wistar rats of 300–350 g were maintained under controlled conditions. A 10 µl drop of the 4-diaminopyrimidines ST-994, ST-1006 and ST-1012 (0.1–10 mM dissolved in normal saline) was applied topically into the lower conjunctival fornix of one eye, either alone or after challenge with the mast cell degranulator C48/80 (100 mg/ml). The contra-lateral eye served as the respective control, while ketotifen was used to validate the experimental model of C48/80-induced conjunctivitis. The animals were sacrificed 20 min after compound administration, the conjunctivae were removed (licence K/2889/11 EL) and HA levels were determined fluorometrically. Topical administration of the H_4R neutral antagonist ST-994 resulted in dose-related increases in the conjunctival HA content, while the partial agonist ST-1006 and the inverse agonist ST-1012 tended to decrease HA levels at high and low doses, respectively. ST-1012 did not reverse the C48/80-induced decreases in the conjunctival histamine content in agreement to the results obtained following topical administration of the H_4R inverse agonist JNJ7777120 in C48/80-induced conjunctivitis. These data point to a constitutive H_4R-meditated automodulatory HA function in the rat conjunctiva. The connection to immediate mast cell degranulation and the elucidation of the components intersecting H_4Rs and automodulatory HA functions under normal and inflammatory conditions is currently under intense investigation.

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A MULTI-APPROACH STRATEGY TO REVEAL THE ROLES OF HISTAMINE (HIS)-RELATED ELEMENTS IN RARE DISEASES

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Alterations in the HIS-related elements have been found in widely spread human pathologies (anaphylaxis and other inflammatory responses, peptic ulcer, neurological and cardiovascular disorders and cancer progression, etc.). For all of these reasons, HIS can be considered the most versatile biogenic amine, playing many different (and sometimes antagonistic) roles in mammalian physiology. Rare diseases (RDs) are very low-prevalence pathologies (<2 patients/1,000 inhabitants). More than 6000 RDs (>20 $\times$ 10^6 patients in Europe) have been described. One of the major problems for RD therapy is the lack of interest to invest in R&D on drugs useful for a small number of patients. Inflammation and neurological abnormalities are commonly included as causes or symptoms of many RDs. The initial hypothesis underlying this work was that HIS must be involved in causes/symptoms of many RDs. In fact, HIS-related genes underlie the most common properties for typical disease related genes. Systems Biology resources, text mining technologies, metabolic and molecular modeling, in combination with experimental validation, provide efficient ways to extract emergent knowledge from the integration of previous fragments of information. By applying this strategy, we are developing several studies based on biocomputational-driven hypotheses with the aim to unveil the putative usefulness of HIS metabolism/signaling modulators in diagnosis, prevention or therapy of these identified RDs. In addition to basic new information on histidine decarboxylase dimerization and its usefulness for selective intervention of inflammatory and neurological RDs, interesting information has emerged on the putative involvement of histamine in several RDs, for instance, Tourette and Sotos syndromes, among others.

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Chemical and genetic aspects of histamine, its receptors and enzymes

HISTAMINE H_4 RECEPTOR: LIGAND, BINDING AND ACTIVATION

H. Stark

The youngest member of the family of histamine receptor subtypes, the histamine H_4 receptor (H4R), has received massive interest due to its influence on inflammatory and immunomodulatory processes.

With respect to drug development some different lead structures have already been described and at least one, perhaps more, have been advanced to clinical trials. Despite this rapid progression there are still some pharmacological tools which are missing since the thus far adopted reference compounds give in different assays conflicting results. This clearly shows that we lack some understanding in the ligand interaction, the efficacy, and the cross-talk in this receptor.

Different classes of H4R ligands will be discussed with an emphasis on the 2-aminopyrimidines which may have the highest number of compounds in this class in connection to strong variance in affinity as well as in efficacy, especially when different species being taken into account. Structurally strongly related 2-aminopyrimidines with comparable affinities, but large differences in efficacies have been investigated in molecular dynamic simulations proposing a different binding mode within this class of compounds depending on their efficacies as partial agonists or inverse agonists.

We propose a "pseudo ionic lock" motif in hH4R that may affect receptor activation, but is not responsible for the high constitutive activity of the receptor. The latter may be caused by a salt bridge between transmembrane domains V and VI which can be seen as a widespread motif in aminergic GPCRs.

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INFLUENCE OF THE LIPOPHILIC PART OF 3-(1*H*-IMIDAZOL-4-YL)PROPYL CARBAMATES ON HISTAMINE H3/H4 RECEPTOR AFFINITY, SELECTIVITY, AND POTENCY

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Human histamine H_4 receptor (hH$_4$R) is the newest histamine receptor and is preferentially active on hematopoietic and immune cells. Due to high structural similarities between H_3R and H_4R it is not surprising, that numerous imidazole-containing H_3R ligands have also significant affinity at the H_4R. Such compounds are considered as new pharmacological tools and could possess therapeutic profile in therapy of inflammatory and immune disorders.

Recently we described branched alkyl, alkenyl and cycloalkyl carbamates of 3-(1H-imidazol-4-yl)propanol that showed both H_3R antagonist/inverse agonist activity and improved H_4R affinity. In expansion of the investigation for histamine H_3 and/or H_4 receptor ligands in this class of compounds and structural requirements for affinity at these receptors, we introduced different substituents in eastern part of molecule. We synthesized series of compounds possessing (un)substituted phenylalkyl or (un)substituted phenoxyalkyl group at carbamate nitrogen.

The compounds were tested for their affinities at hH$_3$R (by competition binding experiments with [^{125}I]iodoproxyfan on membranes of CHO-K1 cells stably expressing hH$_3$R) and hH$_4$R (by [^{3}H]histamine replacement assay on membranes of Sf9 cells, co-expressing hH$_4$R with Gα_{i2} and G$\beta_1\gamma_2$ subunits). Some compounds were also tested for their H_3R potency in vivo by quantification of endogenous N^{τ}-methylhistamine level in the brain of Swiss mice after oral treatment with test compounds.

All tested compounds exhibited good affinity for hH$_3$R with K_i values in range from 30 to 150 nM. Most compounds were also active in vivo. In respect to hH$_4$R affinity in this group of compounds there are some without affinity to this receptor (selective for H_3R) and others with affinity in the micromolar concentration range (Ki = 600–4,868 nM).These results indicated that selectivity for hH$_3$R over hH$_4$R among imidazole-derivatives is possible to achieve.

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COMBINATION OF PROTOTYPICAL HISTAMINE H_3 AND H_4 RECEPTOR PHARMACOPHORES

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

According to the structural requirements responsible for affinity and selectivity for both receptor subtypes, we investigated the combination of 2-aminopyrimidines and related

structures (for H_4R) with the well-established and robust (3-piperidinopropoxy)phenyl pharmacophore (for H_3R). In an effort to design new structurally hybrid compounds, a novel series of substituted pyrimidines were synthesized.

The compounds were prepared by microwave-assisted aromatic nucleophilic substitution reactions of either 2-amino-4,5-dichloropyrimidine or 2,4-dichloropyrimidine. The in vitro receptor binding properties were obtained by radioligand displacement assay on HEK-293 cells stably expressing the hH$_3$R and on Sf9 cells transiently expressing the hH$_4$R (co-expressed with Gαi2 and G$\beta_1\gamma_2$ subunits).

Slight structural changes of the hH$_4$R scaffold evoked severe changes in hH$_4$R affinity, although the 2,4-diaminopyrimidine scaffold can be found in many potent hH$_4$R ligands. In contrast, hH$_3$R affinity has barely been affected by introducing the pyrimidine-related motif on the H_3R pharmacophore. Novel compounds with hH$_3$R binding properties in the nanomolar concentration range have been achieved.

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HIGHLY AFFINITY DIMERIC H_3R LIGANDS INDICATE RECEPTOR DIMERS/OLIGOMERS

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Despite recent progress in clinical trials with hH$_3$R inverse agonists/antagonists, the molecular complexity of H_3R signaling leaves many open questions, e.g. the impact of receptor (homo)dimerization/oligomerization.

We developed a homology model of the hH$_3$R based on the X-ray structure of the hH$_1$R which was then used to build up a hH$_3$R homodimer model taking advantage of the crystallographic X-ray structure of dimeric CXCR4. This model has been the basis for the search of the optimal linker length for divalent H_3R ligands which stabilize dimeric receptors by lipophilic bridging between the pharmacophoric elements within the transmembrane regions. The Asp114 binding pockets have been taken as basic orientation for the measurement of a distance of about 40 Å in pharmacophoric elements. Taking into account some flexibility in the linking chain with different lipophilic transmembrane interactions a spacer of six to 16 methylene units may be acceptable in this model.

Different derivatives of the antagonist JNJ-5207852 have been prepared with different linking elements as we have shown in previous studies that numerous structural variations can be performed maintaining high hH$_3$R affinity. The monomeric precursors have been linked to dimeric compounds by peptide like amide bonds in a tail-to-tail or tail-to-head manner. Most of the di- and structurally related monovalent compounds showed (with a displacement assay) binding affinities in a comparable nanomolar concentration range, but exhibited strong differences in Hill slope, suggesting a different mode/ratio in receptor binding.

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ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR HISTAMINE AND METHYLHISTAMINE DETERMINATION IN TISSUES AND PLASMA

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

16 min, respectively). The most important advantages of U-HPLC are high repeatability and low detection limit, however, sample preparation is time consuming and needs further improvement.

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INTRAVENOUS INFUSION OF ASCORBIC ACID REDUCES PLASMA HISTAMINE LEVELS

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Histamine (HA) plays an important role in allergic and inflammatory diseases. HA intolerance is also an important clinical condition. Degradation of HA in humans occurs through the enzymes histamine-N-methyltransferase (HNMT) and diamine oxidase (DAO).

Ascorbic acid is an essential vitamin and antioxidant agent. An inverse relationship between plasma HA and ascorbic acid concentrations has been found in guinea pigs and test humans.

Test persons (90) were treated with 7.5 g ascorbic acid (Pascoe, Giessen) intravenously over 60 min. Directly before and 1 h after infusion, plasma HA levels and DAO-activity were measured using an ELISA technique (IBL, Hamburg and Sciotech, Tulln).

Plasma HA concentrations were distributed over a range from 0.15 to 0.75 ng/ml. A statistically significant decline (of 34.9 %) was seen in all test persons after ascorbic acid infusion ($p < 0.00005$). After separating test individuals into subgroups with and without allergic diseases, both groups showed a significant decline in HA concentration (no allergy: 30.4 %; allergics: 47.9 %). Interestingly, ascorbic acid had a significantly greater impact on HA concentrations in individuals with allergic diseases ($p = 0.021$) compared to controls. However, DAO levels did not show any difference before and after ascorbic acid infusion (127.74 vs. 110.91 HDU/ml, $p = 0.385$).

This study showed a direct impact of ascorbic acid administration on plasma HA levels. Ascorbic acid reduced plasma HA levels significantly in allergic and non-allergic patients. Patients with allergic diseases showed a statistically greater decline in HA levels. Thus, we can confirm previous results found in guinea pigs and give some objective data for the possible future use of ascorbic acid infusion in the treatment of HA intolerance, mastocytosis, polyvalent allergies, inflammatory disease states etc. Since no change in DAO activity was found, the mechanisms behind the beneficial action of ascorbic acid remain to be further explored.

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HDC-GFP TRANSGENIC MOUSE

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In vivo activity of histamine has been studied well enough to stimulate discussion in the last decade, partly because of the generation of various kinds of gene manipulated mice. On the contrary, the source of histamine in the physiological and pathological situation has not been studied as well, partly because of the lack of useful reporter mice.

We aimed to prepare the reporter mice for histidine decarboxylase (HDC) gene by using a bacterial artificial chromosome (BAC) construct to mimic the expression of the gene. It will reduce the difficulty of gene-expressing cell identification.

We used the longest BAC clone, RP23-40N15, in a library of CHORI for HDC gene. This HDC-BAC clone was homologously recombinated with the plasmid containing the HDC-promoter, GFP gene, and HDC gene without 1st exon, and 3′ region, in *E. coli* strain EL250. Isolated BAC DNA was injected into a fertilized egg to produce a BAC transgenic mouse.

FACS analysis of peritoneal cells of the transgenic mice showed GFP high-expressing cells in the Mac1- and/or c-kit-positive cell fraction indicating that peritoneal macrophages and/or mast cells were positive for GFP expression.

Newly generated BAC-based transgenic mice are promising reporter mice to investigate the transcriptional expression of the HDC gene.

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

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Histamine H_3 receptors (H_3Rs) modulate at the presynaptic level the release of histamine and other

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

The A280 V mutant receptor was generated by overlapping extension PCR amplification using as template the human H_3R of 445 amino acids (hH_3R_{445}) sub-cloned into the vector pCIneo, and verified by automated sequencing. The wild-type and mutant hH_3Rs were stably expressed at similar densities (203 ± 34 and 173 ± 26 fmol/mg protein, respectively, $n = 5$) by CHOK1 cells after lipofectamine-mediated transfection and geneticin-driven clone selection. There were no significant differences between both receptors in their affinities for H_3R ligands ([^{3}H] N-α-methyl-histamine, histamine, R-α-methylhistamine and clobenpropit). The H_3R agonist R-α-methylhistamine inhibited forskolin-induced cAMP accumulation, but the maximum response attained in cells expressing the wild-type hH_3R was significantly larger (students *t* test) than that corresponding to the mutant receptor (-43.3 ± 6.6 and -24.5 ± 2.7 %, respectively, $n = 8$), while the pIC_{50} values were not different (-8.77 ± 0.17 vs. 8.42 ± 0.14).

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

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INTERACTION OF N-METHYL PIPERAZINE DERIVATIVES WITH PTEN PROMOTER

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The aim of this study was the exploration of the interaction of N-methylpiperazine derivatives with the promoter of *PTEN* (phosphatase and tensin homolog deleted on chromosome ten). *PTEN* is one of the most frequently mutated tumor suppressor genes in human cancer, with a frequency approaching that of p53. The protein encoded by *PTEN* is a tyrosine phosphatase. Although it can dephosphorylate proteins, its primary targets are highly specialized membrane lipids, mainly facilitating the removal of the phosphate group from the inositol rings. The examined compounds were synthesized with histamine H_4R targeting activity.

PTEN promoter was amplified together with exon-1 using human genomic DNA that was extracted from blood. Each compound, at a final concentration of 2 mM, was incubated with an equal amount of PCR product at 24 °C for 30 min. Subsequently the mixtures were electrophoresed on 2 % w/v agarose gel.

TR-33 reduced the motility of the PCR product to a greater extent than TR-18 and TR-19. We conclude, that in the group of triazines the substitution of the 3-pyridyl in TR-33 with the 4-b romophenyl in TR-18 reduced the activity. The reduction was even greater when the 2-naphthylmethyl was introduced in TR-19. No effect was observed with TR-20 and TR-28 that differ also with aryl substituent from TR-33. Comparing KB-1, KB-3, KB-8 and KB-28.2 the best result was observed with KB-1 followed by KB-3 and KB-8, while KB-28.2 had no effect. It is interesting to notice that KB-1 and TR-33 possess similar structures with aryl substituent directly connected with triazine core. The best results were obtained among amides with CJ-7, L-58 and L-11. CJ-16, CJ-22 and L-60 had no effect.

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

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

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Human seminal plasma contains considerable amounts of diamine oxidase (DAO). However, information on the function of human seminal plasma DAO, on DAO in seminal plasma from other mammals and on the presence of the second histamine-inactivating enzyme, histamine

N-methyltransferase (HMT), in sperm is scarce. Therefore, we set out to investigate the presence of DAO and HMT in ejaculates from different mammalian species.

Semen samples were collected from pig, cattle, horse, dog and humans and seminal plasma and sperm cells were separated by centrifugation. DAO and HMT activities were determined in seminal plasma from the pre-, main and post-ejaculate and in homogenates of sperm cells using radiometric assays. DAO protein was analyzed in parallel samples by Western blotting with specific antibodies. Total mRNA was prepared from sperm cells and the expression of DAO and HMT was analyzed by RT-PCR using specific primers.

DAO activity was measurable only in human seminal plasma but not in seminal plasma from the other species tested or in sperm cell homogenates. The presence of DAO in human seminal plasma and its absence in the other samples was confirmed by Western blotting with DAO specific antibodies. HMT activity was not detectable in any of the samples analyzed. Neither DAO mRNA nor HMT mRNA was detectable in total sperm cell RNA from any of the species.

From these results we conclude that DAO is present in human seminal plasma but not in seminal plasma from other mammals or in sperm cells. Human seminal plasma DAO is likely to originate from the prostate gland or the testis where DAO expression is detectable at the mRNA level. HMT appears not to be present in mammalian sperm. The presence of DAO only in human seminal plasma is an interesting finding whose functional importance in the fertilization process with respect to histamine and possibly polyamine inactivation remains to be determined.

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Histamine in the cardiovascular and gastro-intestinal systems and cell proliferation and migration

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

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

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

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

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

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

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MAST CELLS, ENDOTHELIAL PROLIFERATION AND CAPILLARY TUBE FORMATION IN MYOCARDIUM OF PATIENTS WITH END-STAGE PRIMARY DILATED OR ISCHEMIC CARDIOMYOPATHY

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Inflammation is an important component in the pathogenesis of many common cardiovascular diseases including ischemic (ICM) and primary dilated (PDCM). The mast cell has established position as a source of inflammatory and pro-proliferative mediators.

The study aim was to examine whether there are differences in the number and distribution of mast cells and in their relation to capillary endothelial proliferation and capillary tube formation in myocardium and subendocardial layer of patients with end-stage PDCM versus ICM.

Myocardial specimens, without degeneration or tissue necrosis, from the anterior wall of 26 heart recipient patients (14 ICM, 12 PDCM) were obtained. H&E staining and immunohistochemistry, using anti-mast cell tryptase and anti-CD31, were employed. Quantitative analysis of the protein expression was performed by the MultiScan Base v.8.08 Image Analysis System. Tryptase-positive mast cells number and area, CD31-positive capillary endothelium area and capillary lumen were estimated in myocardium and subendocardial layer in 65.739 μm^2 microscopic field. Values are presented as mean $\pm$ SD. For statistical evaluation, U Wilcoxon–Mann–Whitney test and Spearman range correlation coefficient were applied.

In myocardia of patients with end-stage PDCM, in comparison with ICM patients, an increased numerical density (0.51 ± 0.14 vs. 0.32 ± 0.16) and area (44.33 ± 15.41 vs. 29.71 ± 13.98 μm^2) of mast cells and excessive capillary endothelial cells proliferation with defect of capillary tube formation, respectively, was found. Microvessel lumen and endothelium areas quotients were in PDCM always below 1 (0.53 ± 0.26), while above 1 (2.79 ± 1.53) in ICM patients. CD31-positive endothelium area correlated with tryptase-positive mast cell area in both groups. No differences were found in mast cell density in subendocardial layer.

This preliminary study suggests that the mast cells in myocardium may be responsible for excessive capillary endothelial proliferation in tandem with defects of capillary

tube formation, as expressed by undeveloped lumen in primary dilated versus ischemic cardiomyopathy patients. These observations may have therapeutic significance.

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

J. Jochem

Thyrotropin releasing hormone (TRH), apart from the role in hypothalamus-pituitary-thyroid axis, influences many functions of the central nervous system, including cardiovascular regulation. Intravenously (iv) administered TRH evokes the resuscitating effect in haemorrhage-shocked rats, with increases in mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). The mechanism of the effect is associated with the activation of brain cholinergic neurons and central muscarinic receptors. Interestingly, recent in vitro studies by the group of Haas and Sergeeva demonstrate excitation of histaminergic neurons of the tuberomamillary nucleus (TMN) by TRH. Since we revealed previously the resuscitating effect resulting from central histaminergic system activation, and immunohistochemical analysis showed TRH type 2 receptors within TMN neurons, the aim of this study was to examine an involvement of the histaminergic system in TRH-evoked effects in haemorrhagic shock. Experiments were carried out in ketamine/xylazine-anaesthetised male Wistar rats (n = 6 in all groups), subjected to haemorrhagic hypotension, with MAP stabilized at 20–25 mmHg, which resulted in the death of all control animals within 30 min. TRH (3 mg/kg) given iv at 5 min of critical hypotension evoked long-lasting increases in MAP, PP and HR, with a 100 % survival at 2 h. Haemodynamic effects observed 40 min after treatment, but not the survival rate at 2 h, were significantly decreased by intracerebroventricular pretreatment with histamine H_1 receptor antagonist chlorpheniramine (50 nmol), but not with ranitidine (50 nmol), clobenpropit (42.5 nmol) and JNJ 10191584 (25 nmol)—H_2, H_3 and H_4 receptor antagonists, respectively. Thus, these results demonstrate for the first time the involvement of the histaminergic system in TRH-induced resuscitating effect in haemorrhagic shock in rats.

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

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

First, we found that histamine H_3 receptors (H_3R) are expressed on rodent and human pancreatic β-cells. Next, we examined the role of H_3R in glucose-induced insulin secretion (GIIS) from MIN6 cells, a cell line derived from mouse pancreatic β-cells. The inhibitory effect of H_3R on GIIS was assessed pharmacologically using the specific agonist, imetit. H_3R signaling inhibited insulin granule exocytosis, the final step in GIIS, but did not affect upstream events in GIIS such as increases in intracellular ATP and Ca^{2+} concentrations.

We found that the activation of H_3R with imetit also attenuated BrdU incorporation in MIN6 cells by 33 %. We examined which factors were involved in the H_3R-mediated inhibition of β-cell proliferation. Imetit decreased the phosphorylation of cyclic AMP response element binding protein (CREB), one of the essential transcriptional factors for β-cell proliferation. Thus, H_3R signaling likely regulates β-cell proliferation by decreasing CREB phosphorylation.

Overall, these lines of evidence suggest that H_3R expressed in β-cells may play a pivotal role in energy homeostasis by regulating insulin secretion and β-cell proliferation. Therefore, H_3R could be a diagnostic and/or therapeutic target in diabetic patients.

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

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Mast cells (MC) and interstitial cells of Cajal (ICC) develop close spatial contacts in the gastrointestinal (GI) tract. Intestinal motility disturbances are frequently associated with a reduced number of ICC as well as with

immune cell infiltration. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is an endogenous agent primarily formed from aromatic amine precursors by an enzymatic or a non-enzymatic pathway leading to neurotoxic N-methyltetrahydroquinolinium ions, which may play a role in the etiology of Parkinson's disease (PD). Gastrointestinal dysmotility observed in PD patients may partly be due to peripheral effects of salsolinol. The aim of this study was to evaluate the chronic effects of salsolinol on MC and ICC in the jejunum of rats.

Salsolinol (200 mg/kg in total) was continuously administered to male Wistar rats using intraperitoneal ALZET (Durtec, USA) osmotic mini-pumps for 2 weeks (delivery rate 0.5 μL/h, $n = 4$) or 4 weeks (delivery rate 0.25 μL/h, $n = 4$). Sham animals implanted with mini-pumps delivering saline ($n = 8$) served as the controls. At the end of the experiment, animals were decapitated and segments of jejunum were removed. Longitudinal muscle–myenteric plexus preparations (LMMP) were double-stained with anti-c-Kit polyclonal antibody (Dako, USA) and anti-tryptase polyclonal antibody (Dako, USA).

The number of cells stained with both anti-c-Kit and anti-tryptase (i.e., MC) in the GI wall was decreased in both salsolinol-treated groups compared to the control group. However, the number of degranulated MC was elevated in both salsolinol-treated groups compared to the control group. The number of cells stained with anti-c-Kit only (i.e., ICC) was lower in both salsolinol-treated groups in comparison with the control group.

In our model, chronic, intraperitoneal administration of salsolinol damages MC and ICC. We speculate that this may lead to an impairment of GI function.

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

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We have recently reported that experimental periodontitis reduced methacholine-induced submandibular gland (SMG) salivary secretion. The aim of the present study was to determine whether histamine could prevent SMG impairment produced by experimental periodontitis. Bilateral experimental periodontitis was induced in male rats by placing a cotton thread ligature around the neck of both first lower molars for 2 weeks; and histamine treatment (0.1 mg/kg, s.c.) was initiated 5 days before the end of the experimental period. The effects of histamine on periodontitis-altered functional and histological parameters of SMG and on periodontal bone loss were evaluated in 48 animals ($n = 12$ each group).

Histamine treatment partially reversed the reduction in methacholine-induced salivation produced by experimental periodontitis while preventing SMG histological damage. The effect of histamine on SMG was associated with an increased proliferation rate (2.2 ± 0.3 vs. 0.2 ± 0.2 proliferative cells per field evaluated by PCNA immunoreactivity, $P < 0.001$). Furthermore, histamine completely prevented the increase in apoptosis associated with experimental periodontitis (1.0 ± 0.4 vs. 60.9 ± 4.6 apoptotic cells per field evaluated by the TUNEL assay, $P < 0.001$). The protective effect of histamine on SMG functionality was associated with attenuation of lingual and vestibular bone loss (0.66 ± 0.04 vs. 0.97 ± 0.06 mm; $P < 0.001$) and a reduction of increased levels of prostaglandin E_2 in gingivia of rats with experimental periodontitis.

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

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RADIOPROTECTIVE POTENTIAL OF HISTAMINE ON RAT SMALL INTESTINE AND UTERUS

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Based on our previous data which showed the histamine radioprotective effect on mouse small intestine, bone marrow and salivary glands, in the present work we aimed to improve our knowledge about the histamine radioprotective potential by investigating its effect on reducing ionising radiation-induced injury in the rat small intestine and uterus. Rats were divided into 4 groups. Histamine and histamine-5 Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg) starting 24 h before irradiation. Histamine-5 Gy and untreated-5 Gy groups were irradiated with a dose of whole-body Cesium-137

irradiation. Three days post irradiation animals were sacrificed and tissues were removed, fixed, stained with haematoxylin and eosin and histological characteristics were evaluated. Proliferation and apoptosis markers were studied by immunohistochemistry. Results demonstrate that histamine treatment reduced radiation-induced mucosal atrophy, oedema and vascular damage produced by ionising radiation, increasing the number of crypts per circumference (239 ± 12 vs. 160 ± 10, $P < 0.01$). This effect was associated with a reduction of radiation-induced intestinal crypts apoptosis. Furthermore, radiation-induced flattening of the endometrial surface, depletion of deep glands and reduced mitosis, effects that were completely blocked by histamine treatment. The expression of a proliferation marker in uterine luminal and glandular cells was markedly stimulated in histamine treated and irradiated rats. The evidence indicates that histamine is a potential candidate as a safe radioprotective agent that may increase the therapeutic index of radiotherapy for intra-abdominal and pelvic cancers. However, its efficacy needs to be carefully investigated in prospective clinical trials.

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

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We have previously demonstrated that cell migration and invasion of human breast carcinoma MDA-MB-231 cells were stimulated by low doses (0.5–1 μM) of histamine (HA), while inhibited by concentrations greater than 10 μM. These opposite actions are mediated by different intracellular hydrogen peroxide (H_2O_2) levels. *c-Src* protein is activated by phosphorylation and this is critical for cell migration and cancer progression to metastasis. This protein phosphorylates substrates such as beta-catenin which translocates to the nucleus and modulates the expression of genes related to the epithelial mesenchymal transition (EMT). The aim of this work was to study the role of the *c-Src* protein in HA-induced migratory response of MDA-MB-231 cells.

We used a *c-Src* specific inhibitor, PP2, in a concentration which had no effect on cell proliferation. 1 μM PP2 blocked the stimulatory action of low doses of HA in cell migration and invasion as determined by transwells units coated or not with matrigel®. PP2 also blocked increase in further EMT markers (gelatinolytic activity and alpha smooth muscle actin expression) induced by 1 μM HA. An increase in *c-Src* phosphorylation was observed by western blot with 1 μM HA or exogenous 0.5 μM H_2O_2 while a decrease was registered when HA was >10 μM or 5 μM H_2O_2 were used. Catalase treatment reversed those effects.

The expression of beta-catenin protein was evaluated by immunostaining and Western blot. There was a higher nuclear and perinuclear expression with 1 μM HA. Conversely, the combined treatment of 1 μM HA plus 1 μM PP2 produced a cytoplasmic and nuclear beta catenin expression similar to non-treated cells. Low doses of HA also inactivated GSK-3beta, an enzyme involved in cytoplasmic degradation of beta catenin, favoring its nuclear translocation.

In summary, our results indicate that HA modulates the migratory and invasive capacity of MDA-MB 231 cells through a singular GPCR signaling pathway involving a H_2O_2-induced *c-Src* phosphorylation.

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

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Melanoma accounts for less than 5 % of skin cancer cases but causes a large majority of skin cancer deaths. The expression of all histamine receptor (HR) subtypes was demonstrated in human melanoma cell lines, and the activation of H_4R in WM35 primary and M1/15 highly metastatic human melanoma cells inhibits proliferation and migration, and induces differentiation and senescence. The aim of this work was to evaluate the in vivo anti-tumour potential of histamine (HA) and clozapine (CLZ, H_4R agonist) on human melanoma. An experimental model was developed by subcutaneous (sc) injection of M1/15 cells into the right flank of athymic nude mice. Animals were separated into three groups: control, HA (1 $mg.kg^{-1}$, sc) and CLZ (1 $mg.kg^{-1}$, sc). After spontaneous death, tumours were excised and the expression levels of H_4R, HA and histidine decarboxylase (HDC) were studied by immunohistochemistry. Cell growth was assessed by PCNA expression and by mitotic index (MI). Finally, vascularization was determined by Massons trichromic

staining and invasion markers by metalloprotease (MMP-2 and MMP-9) expression levels. Mice receiving HA or CLZ showed a median survival increase (61 days) verus control group (40 days) treated with saline solution ($p < 0.05$). Tumour volumes after 70 days of HA (2.7 cm^3) or CLZ (3.0 cm^3) treatment were significantly lower than in control mice (12.8 cm^3) ($p < 0.01$). Treatment with HA (12.8 ± 4.3 days) or CLZ (13.8 ± 4.3 days) increases tumour doubling time versus control group (5.0 ± 1.1 days), $p < 0.05$. PCNA, MI, intra-tumoral neovascularization and the expression of MMP-2 and MMP-9 were diminished in HA and in CLZ treated mice, while the expression of H_4R, HA and HDC seemed not to be modified. We conclude that HA and CLZ show an anti-tumour effect in human melanoma. Further studies are needed to corroborate the importance of H_4R as a potential target for new drug development for the treatment of this disease.

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HISTAMINE INACTIVATING ENZYMES IN BREAST CANCER CELL LINES

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There is increasing evidence to demonstrate that histamine plays a significant role in breast cancer progression since functional histamine receptors and L-histidine decarboxylase activity are present in breast tissue. In addition, histamine is increased in plasma and cancerous tissue derived from breast cancer patients compared to healthy subjects. As the importance of histamine catabolism has not been fully studied in breast cancer cells, the aim of the present work was to investigate the expression and activity of the histamine inactivating enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HMT) in two breast cancer cell lines (MDA-MB-231 and MCF-7) with different malignant characteristics. Furthermore, we evaluated whether histamine treatment could modulate cell expression and activity. The expression of these two enzymes was determined by RT-PCR and western blot while the activity was measured by a radiometric assay. Results show that DAO was not detected in either cell line either at the mRNA or protein level. However, HMT enzyme was expressed in MDA-MB-231 and MCF-7 cells. In agreement with these results, DAO enzyme activity was not observed while HMT activity was detected in both cell lines. Histamine induced a down-regulation of the HMT activity, exhibiting a threefold decrease in MDA-MB-231 and MCF-7 cells ($P < 0.0001$). We conclude that HMT is the only histamine inactivating enzyme detected, suggesting a crucial role of this enzyme in histamine catabolism in breast cancer.

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

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We have previously demonstrated a high expression of histidine decarboxylase (HDC), the enzyme involved in histamine (HA) synthesis and therefore a high HA content in the human pancreatic carcinoma cell line BxPC3. In vivo, HA produced a significant desmoplasia in nude mice bearing BxPC3 xenografts and also neoplastic cells colonizing lung parenchyma were observed. In vitro, conditioned medium of BxPC3 cells treated with HA (1–10 μM) increased the number of CCD-1059Sk cells in S phase of the cell cycle. The aim of this work was to evaluate HA action on cell proliferation, gelatinase expression and activity and cell motility in the normal human fibroblast cell line CCD-1059Sk. A direct action of HA (1–10 μM) on fibroblast proliferation was observed employing a bromodeoxyuridine (BrdU) incorporation technique. After 8 h of HA treatment there was a significant rise in number of fibroblast cells in S phase ($p < 0.05$ vs. non-treated cells). By RT-PCR, we detected mRNA expression of H_1, H_2 and H_4 histamine receptors, the matrix metalloproteinase 2 (MMP2) and the tissue inhibitor of MMP (TIMP1) in this cell line. Immunostaining studies showed that CCD-1059Sk fibroblasts expressed cytoplasmic MMP2 protein, although no difference in MMP2 expression was observed between HA treated and non-treated cells. Gelatinolytic activity evaluated by zymography was significantly increased by 10 μM HA (195 % vs. control, $p < 0.001$) and reproduced by 10 μM H_1 histamine receptor agonist 2-((3-trifluoromethyl)phenyl) histamine dimaleate (190 % vs. control, $p < 0.001$). 10 μM HA enhanced cell migration ($p < 0.05$ vs. non treated cells) assessed using transwell units. In summary, we may conclude that exogenous HA exerts a direct action on CCD-1059Sk cell proliferation,

gelatinolytic activity and motility, opening the perspective for future studies on the role of histamine in fibroblast activation during the epithelial mesenchymal transition related to cancer progression.

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

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Our previous data suggested an allosteric action of histamine to modulate cAMP formation induced by the activation of β_2 adrenoceptors endogenously expressed by human prostate cancer cells DU-145. In this work we have studied further this issue by combining functional assays with heterologously expressed human β_2 adrenoceptors (hβ_2-ARs) and molecular modeling.

In COS-7 cells transiently transfected with the hβ_2-AR (10.4 ± 1.1 pmol/mg protein), incubation with histamine had no effect on basal cAMP accumulation but enhanced the response to the β-AR agonist isoproterenol (153 ± 15 % of control response) in a concentration-dependent manner (EC_{50} 6.2 μM, pEC_{50} 5.21 ± 0.24). In contrast, the inhibition by isoproterenol of [^{3}H]-dihydroalprenolol binding was not modified by histamine (p*K*i 5.99 ± 0.20 and 5.95 ± 0.10 in the presence and the absence of 10 μM histamine, respectively). Modeling on the hβ_2-AR showed the presence of three sites capable to bind imidazol-contaning drugs, and two of these sites had theoretical affinities (2–3 μM) in the range of histamine modulation of cAMP accumulation observed in transfected cells.

Taken together, these data provide further support for an allosteric action of histamine to modulate the intracellular signaling of human β_2 adrenoceptors.

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