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The European Histamine Research Society 44th Annual Meeting, May 6–9, 2015 Malaga, Spain

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Meeting Report of the European Histamine Research Society

G. Sturman

This year's meeting was in Torremolinos in the Malaga Province of Spain at the kind invitation of Professor Francisca Sánchez Jiménez or Kika as we know her. This is the second time that histaminologists have met in Torremolinos; the previous meeting in 1992 was organised by Manuel Garcia-Caballero. This year's meeting was held in the Hotel Amaragua, which is situated beside the beach and in beautiful surroundings. Torremolinos was initially a small fishing village which grew rapidly during the second half of the 20th century as a consequence of the tourism boom on the "Costa del Sol" and is now an important part of the financial heart of Southern Spain.

This year there were over 80 people registered and they represented nearly 20 countries (mostly from Europe but also from North, South and Central America, Japan as well as some other Middle Eastern countries). Some regular attendees could not attend and they were missed but a big welcome was made to all the new visitors, who we hope will return to future meetings. Most of the delegates arrived on the Wednesday. The Council met as usual late afternoon. Then there was the Welcome Reception in the hotel gardens which was sponsored by Ciberer. Here everyone was able to greet old friends as well as make new ones whilst we nibbled on canapés and drank Spanish wines and sherry—a good start to the meeting.

Thursday started with the Opening Ceremony for the 44th meeting of our society and we were welcomed by our President, Paul Chazot and our host Kika, who thanked all the helpers including the hotel staff. She also reported that there had been 66 abstracts submitted giving us 7 oral sessions, 3 poster sessions and also 5 invited speakers. This was followed by the Vice Rector of the University of Malaga, Professor Victor Muńoz who welcomed us and wished us a very successful meeting. Then we paid our respects to 2 of our members who had very sadly died during the year; Professors Wilfried Lorenz (one of our Honorary Members) from Germany and Yrjő Konttinen of Finland. Paul then gave out the student bursaries certificates and €500 for each of the 11 student members. The El-Sayed Assem family very kindly sponsored two students while the rest were sponsored by our society. Then we had the Honorary Membership ceremony where Professor Emanuela Masini of Florence University was presented with her official certificate beautifully written in Latin and sporting the society's official seal. Emanuela when thanking the society for this honour also thanked another of our Honorary Members, Piero Mannioni who had allowed her to think freely and develop her ideas while she worked in his department. Then we started with the scientific programme and Bernie Gibbs introduced Dr Gunnar Pejler of Uppsala University, Sweden, who is a pioneer in mast cell science. This was the first of the plenary lectures and was entitled 'Mast cell granules: armed with histamine and friends' and described the positively and negatively charged molecules which are important in the storage process in mast cells. After the coffee break, which was on the hotel terrace, we listened to the first oral session which was a Round Table—IUPHAR Immunopharmacology Section on 'Histamine and the immune system: Pros and Cons.' Then we had a group photo taken and lunch. During the lunch, the Early Stage Researchers met with one of the Council Members (Astrid Sasse) to talk to about their ideas/wishes for the society.

After lunch we were back to listen to the next plenary lecture which was introduced by Kika and delivered by Dr Federico Mayor Menéndez of the Universidad Autónoma de Madrid, Spain. It was entitled 'G protein-coupled receptor kinases: from GPCR modulation to signalling hubs'. This was followed by the second oral session which was on Molecular Pharmacology and we heard 4 very interesting presentations. Then we moved to a nearby room to listen to the poster presentations and read of the posters. In the evening after dinner, many of us listened to the impromptu and delightful piano playing of Roman with help from Friedhelm.

The GB West Memorial Lecture was scheduled for first thing on Friday. Beatrice Passani (University of Florence) gave the introduction to Professor Emanuela Masini. This lecture was entitled 'Histamine and Relaxin: an intriguing connection'. At the end of this very interesting lecture, Emanuela was presented with a copy of GB West's autobiography—'A Handful of Luck'. The next oral presentation session (Histamine and Sleep) was then given before the coffee break. When we commenced again, we listened to the fourth oral session communications which were on 'Histamine and Neurobiology.'

After this session we then went by 2 coaches towards Malaga and then we headed first north and then west. Each coach had a guide who explained how the area developed from being a trading port for olive oil, wine and fish to being

the capital of Spanish tourism in southern Spain with the third largest Spanish airport. We drove through the Andalucían countryside, through vineyards, orange and olive groves to Ronda which is a town 100 km from Malaga. Ronda is situated in a very mountainous area about 750 m above sea level. The Guadalevín River runs through the city, dividing it in two and carving out the steep, 100 plus meters deep El Tajo canyon upon which the city perches. Ronda's Romero family played a principal role in the development of modern Spanish bullfighting. On leaving our coaches we then walked through the 'new town' to a restaurant opposite the bull ring. At the restaurant we had an enormous meal in which we had what seemed like a whole leg of lamb each! Then with guides we were able to explore both the new and old towns of Ronda which are linked by a bridge which spans this very deep gorge and could marvel at the views of the surrounding countryside. In the old town the street were very narrow but full of character. Continuing on our coach outing, we headed back through the mountains towards the coast stopping to see the rock of Gibraltar in the distance. Our destination was Puerto Banús which was built in 1970 by José Banús, a local property developer, and has since become one of the largest entertainment centres in the Costa del Sol, with 5 million annual visitors, and is popular with international celebrities. The focal point of Puerto Banús is the marina while its streets are lined with expensive luxury boutiques. On returning to Torremolinos we went to the Patati Restaurant on the beach where we had a family-style meal with typical Spanish tapas and fish. We were then treated to a flamenco dancing display in our hotel.

The following day the meeting started with a plenary lecture given by Professor Rob Leurs (The Netherlands) entitled 'Towards third generation antihistamines: fact or fiction?' He was introduced by Pertti Panula (Helsinki, Finland) and we learnt about the development of new antihistamines from the molecular analysis of the various histamine receptors. This was followed by a session in memory of Professor Wilfred Lorenz, an Honorary Member of our society who tragically died last year. It was entitled 'Histamine, as a regulator of cell proliferation, differentiation and communication: From the molecular bases to applied biomedicine.' This session was introduced by Madeleine Ennis, who gave a very moving tribute to Wilfred. After this session there was a further viewing of the best 7 posters as judged by the poster jury. In the first session in the afternoon we listened to our younger members (PhD students or not more than 3 year's post-doctoral research) who had been selected to give presentations for the EHRS Young Investigator Award (YIA). This competition was sponsored by Janssen Pharmaceutical Company. It was another difficult task for the judges to differentiate between these six excellent presentations. The winner was Fumito Naganuma (Sendai, Japan) for his presentation entitled 'Analysis of histamine *N*-methyltransferase deficient mice.' The rest of the finalists were given Highly Commended Certificates. The final oral session of the meeting was entitled Biogenic Amine Network where there were 4 presentations from the Malaga group on aspects of the structures and genes of histaminergic enzymes and histamine receptors.

Throughout the meeting the poster committee (Arianna Rosa, Cecilia Lanzi, Vanina Medina, Mariaconcetta Durante, Ignacio Fajardo and Eléonore Verweij) 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 Daniel McNaught-Flores et al., from Amsterdam, The Netherlands with the poster entitled 'Pharmacological characterization of Zebrafish H_3R-like receptors', second to Laura Lucarini et al., from Florence, Italy with the poster entitled 'Is PARP modulation involved in histamine H_4R signalling in an inflammatory model of lung fibrosis?' and third prize went to Diego Martinel Lamas et al., from Buenos Aires, Argentina with his poster entitled 'Increasing radiosensitivity with histamine in 1205 Lu human melanoma cells.' The authors of the four other posters short listed were given Highly Commended Certificates.

After this we held our General Assembly where the new Council was elected and they are Vanina Medina (Argentina), Saara Nuutinen (Finland), Arianna Rosa (Italy) and Ekaterini Tiligada (Greece) and Paul Chazot was re-elected as President. This was followed by our traditional Farewell Dinner with the Award Ceremony with certificates and prizes being given out. All YIA finalists and poster prize winners received a souvenir from the organising committee. Then as usual we had our singing session, beginning with "Anita's Thank You Song" (sung to the tune of 'Clementine') as a big thank you to Kika and her team for the excellent and memorable meeting. After this we sung our EHRS Anthem, before saying 'au revoir' to our many 'histaminergic' friends.

Our thanks are given to all of the Spanish histaminologists for the excellent meeting. The next meeting will be held in Florence, Italy (11–14 May, 2016) at the kind invitation of Emanuela Masini and the Florentine pharmacologists.

Participants of the 2015 meeting of the EHRS.

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Honorary Memberships in the European Histamine Research Society

B. Passani, 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 13 current Honorary Members of the Society; Madeleine Ennis, Agnieszka Fogel, Robin Ganellin, Helmut Haas, Zsuzsanna Husti, Piero Mannaioni, Bruno Mondovi, Fred Pearce, Jean-Charles Schwartz, Henk Timmerman, Ingrid Olhagen-Uvnäs, Takehiko Watanabe and Jean West. Former Honorary Members of this society include Sir James Black, Franc Erjavec, Wilfried Lorenz, Czeslaw Maslinski, Wolfgang Schmutzler, Bőrje Uvnäs and Geoffrey West. At this meeting, the society awarded Honorary Membership to a special person who has all contributed significantly to the EHRS over the years and she is Professor Emanuela Masini of University of Florence, Italy.

The oration for Emanuela was given by one of her colleagues, Beatrice Passani, who said that it was an honour and pleasure to share part of her scientific life with Emanuela, who would give advice, suggestions and scathing comments which always contributed the quality of Beatrice's work. Beatrice said that writing about Emanuela Masini was easy if you confined yourself to the list of Emanuela's many scientific and professional achievements. She has co-authored over 200 high profile papers published in international peer-reviewed journals, and at least a 100 of them carry the word *histamine* in the title. She is Professor of Pharmacology at the University of Florence, Director of the Toxicological Unit at the Careggi Hospital in Florence, Director of the Centre for Higher Education on Environmental Risks and Prevention and of the School of Medical Toxicology. But there is much more to be told about Emanuela Masini. Her curiosity and insatiable eagerness to explore new scientific venues and implement new experimental protocols has always been contagious among her collaborators, both with the young and more experienced ones. Always supportive of her doctoral students, she always would give words of encouragement when failure strikes in the laboratory or when personal problems arise.

A truly talented lady, Emanuela Masini has a flair for the unusual and used her scientific knowledge to other ends, writing two interesting and 'enticing' books *Cioccolata, alimento del gusto, della salute e del piacere* (*Chocolate, a nourishment for taste, health and pleasure*) and *Te', bevanda euforizzante, infuso terapeutico, disciplinato piacere* (*Tea, a euphoric drink, a therapeutic infusion and disciplined pleasure*). Last but not least, Emanuela is a regular member of our society and has hosted a meeting in 2007 and will be the hostess for next year's meeting. She rightly deserves being elected an Honorary Member of the European Histamine Research Society.

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Professor Emanuela Masini and Paul Chazot, President of the EHRS

Immunological aspects of histamine

MAST CELL GRANULES: ARMED WITH HISTAMINE AND FRIENDS

G. Pejler

Mast cells (MCs) are myeloid cells derived from hematopoietic stem cells of the bone marrow. They circulate as immature progenitors in blood, from which they home to most tissues of the body and there develop into mature MCs by the influence of local growth factors such as stem cell factor and IL-3.

The most clearly distinguishing feature of MCs is their remarkably high content of highly electron-dense secretory granules. The granules are filled with a number of preformed compounds ("mediators"), including bioactive amines (histamine, serotonin, dopamine, polyamines), preformed cytokines (e.g. TNF), proteoglycans of serglycin type and MC-restricted proteases. Serglycin is composed of a small protein "core", to which highly sulfated and thereby negatively charged glycosaminoglycans of either heparin or chondroitin sulfate type are attached, and previous studies have demonstrated a crucial role for serglycin in promoting the storage of histamine, serotonin and the MC-restricted proteases. The MC-restricted proteases encompass serine proteases of chymase and tryptase type, which both are endopeptidases, as well as carboxypeptidase A3, the latter an exopeptidase belonging to the metalloprotease family.

Studies imply that granule homeostasis is a result of a dynamic electrostatic balance between granule compounds of opposite electric charge. Hence, a reduction in negative charge (as imposed by the absence of serglycin or N-deacetylase/N-sulfotransferase-2) results in a decrease in the ability of granules to accommodate positively charged compounds. Conversely, a reduction of positively charged compounds (as imposed by the absence of proteases or histamine) results in a corresponding decrease in the ability of granules to accommodate negative charge. In addition to these electrostatic effects described above there are also indications of homeostatic mechanisms that are not necessarily of electrostatic nature.

MCs are activated during various pathological conditions. A hallmark event during such circumstances is that MCs undergo degranulation, by which the preformed granule compounds are released to the exterior. MC activation can be induced by many mechanisms, of which binding of multivalent antigen (allergen) to IgE molecules bound to their high affinity cell surface receptor (FcεRI) represents the "classical" MC activation that is a hallmark event during allergic responses. However, MCs can be activated by numerous other mechanisms, including stimulation by anaphylatoxins, neuropeptides and various toxins, as well as through engagement of toll-like receptors.

Undoubtedly, MCs are mostly known for their detrimental impact on various allergic conditions including allergic asthma. However, MCs have, during more recent years, been recognized for having a detrimental impact on a panel of additional disorders, including cancer, atherosclerosis, arthritis, aneurysm formation, diabetes, fibrosis and obesity. Conversely, MCs have also been implicated to carry beneficial functions, most notably in the context of bacterial infection.

Having identified a role for MCs in a variety of pathological settings, a major focus for current research is to gain a deeper understanding of the molecular mechanisms by which MCs influence a given pathological condition. Recent studies have shown that, in many cases, the effects of MCs on an immune reaction are closely associated with the biological actions of the released preformed granule compounds, as exemplified by recent findings showing that MC granule amines and proteases account for many of the protective and detrimental effects of MCs in various inflammatory settings.

Based on these findings, another major focus for MC researchers is to find ways to intervene with MC-mediated pathological events, as exemplified by the use of histamine receptor antagonists and various inhibitors of MC-restricted proteases. A novel approach for this purpose is to selectively induce MC apoptosis by permeabilization of their secretory granules. We have recently developed this concept and have shown that this strategy is an effective means of selectively depleting MCs both in vitro and in vivo. Recently, we have also shown that one of the MC granule compounds, tryptase, in addition to being released to the exterior can be found in the nuclear compartment of MCs. Here, tryptase has been found to cause truncation of core histones, thereby removing sites for epigenetic histone modification.

In this presentation, novel aspects of MC granule function were discussed.

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ROLE OF HISTAMINE IN IMMUNOLOGICAL AND ALLERGIC PROCESSES

M. Blanca, J. A. Cornejo, J. Perkins, M. J. Torres

Since the physiological and pathological effects of histamine were first described, there has been impressive research on this molecule. Histamine is synthesized by many cells from the precursor histidine. The production is regulated at the transcriptional and translational level by histidine decarboxylase. Mast cells and basophils store

histamine in their cytoplasmic granules. Histamine acts through its receptors on different cells of the immunological system. Via the H_1R receptor, it acts on T helper cells (TH1) by inducing proliferation and production of interferon gamma and by the H_2R on TH2 cells by decreasing IL-4 and IL-13. H_4R are present on monocytes, natural killer cells and dendritic cells. Histamine increases the chemotactic activity of NK cells via the H_4 receptors.

Histamine is a mediator in hypersensitivity drug reactions that include those mediated by specific immunological mechanisms and others without the demonstration of a specific immunological mechanism. IgE mediated and T cell effector cell dependent are the most common. IgE mediated reactions include anaphylactic shock, urticaria and angioedema. T cell mediated reactions include exanthema, urticaria, fixed drug eruption, acute exanthematic pustulosis, DRESS syndrome and toxic epidermal necrolysis. In these reactions there is a component of erythema and pruritus in the skin induced by histamine. In other entities histamine can be released in the absence of a specific immunological mechanism. The most common is urticaria and angioedema induced by non steroidal anti-inflammatory drugs (NSAIDs).

The parallel monitoring of tryptase in peripheral blood and N-methyl histamine in urine enables the diagnosis of anaphylactic drug reactions. Histamine is also an important mediator in the so called accelerated reactions to drugs, which in fact belong to a T cell effector response. It can be also quantitated in the nose after challenge with an appropriate allergen in subjects sensitised to pollens as well as other allergens.

Our group is very interested in histamine pharmacogenetics. We have shown that certain genetic variants in the diamine oxidase gene are associated with hypersensitivity reactions to NSAIDs as well as a variability of the L-histidine decarboxylase gene in allergic rhinitis.

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HISTAMINE IN IMMUNOPHARMACOLOGY: PROS AND CONS

E. Tiligada

In recent years, several notable changes in our understanding of the immune system and the biotechnological advances have made new classes of clinically relevant drugs available, including monoclonal antibodies and kinase inhibitors that selectively modulate immune cell subsets. Despite the emergence and the clinical success of biologics, numerous limitations hamper the therapeutic manipulation of the inflammatory networks underlying the multifaceted aetiology of many immune disorders. Therefore, by controlling signaling pathways implicated in tissue-specific inflammation, small molecules remain an effective approach in immunomodulatory drug development and repurposing. The latest developments in histamine research illustrate the challenge to identify and validate new targets and to optimize lead candidates for the treatment of many forms of highly prevalent and debilitating diseases. In addition to the continuing appreciation of the pharmacological diversity of antihistamines targeting the H_1 receptor, the discovery of the high affinity H_4 receptor revealed new concepts in the extensive biological functions of histamine and exposed attractive perspectives for the therapeutic potential of this new drug target in acute and chronic inflammation, host defense and neuropathic pain. Further to the rapid entry of H_4 receptor-targeting compounds into advanced clinical development, the pluridimensional pharmacological efficacy of H_4 receptor ligands exhibiting biased agonism suggest that the differential expression and/or the selective modulation of pro- and anti-inflammatory signals orchestrates acute and chronic inflammation reflected by the repertoire of immune cells and mediators. The ongoing efforts focus on the assessment of translating preclinical data into promising therapies for pathologies with high economic and societal impact, such as allergies and autoimmune diseases.

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MODULATION OF THE IMMUNE RESPONSE IN ASTHMA BY THERAPEUTIC ANTIBODIES

M. Ennis

Asthma is a chronic inflammatory disease of the airways in which many cells and their mediators play a role. Current treatment with inhaled β2 agonists and corticosteroids is usually effective for most people with mild/moderate disease. However, some patients with severe asthma are poorly responsive to steroid treatment. These patients, as well as having a poor quality of life, also place a significant burden on the Health Service. This has led to the search for other treatment modalities. To date, only Omalizumab has been licensed for use in asthma. It is a monoclonal antibody directed against IgE. In patients, it lowers circulating IgE as well as the number of IgE positive cells in the airways. It also reduces the number of days with exacerbations and patients can reduce the amount of inhaled

steroids that they use. However, it is not effective in all patients with severe asthma. New anti-IgE directed antibodies are being developed and tested. This has led to the study of other therapeutic antibodies which are currently in various stages of clinical investigation. These include antibodies directed against IL-5 and the IL-5 receptor, IL-4 and the IL-4 receptor α, IL-13 and TNFα and its receptor. These agents are currently in various phase II and III clinical trials. Clinically there have been mixed reports on the efficacy of the tested agents, reflecting the heterogeneous nature of asthma. Further work into careful stratification of patients is necessary.

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CORRELATION OF SERUM HISTAMINE AND SPECIFIC IgG4 LEVELS AS AN INDICATOR OF TOLERANCE IN ALLERGIC INDIVIDUALS

C. Chliva, X. Aggelides, M. Makris, E. Tiligada

Natural high dose exposure of beekeepers to honey bee venom (HBV) is a valuable model to dissect the mechanisms of tolerance to allergens. Reliable biomarkers for the tolerant phenotype are lacking. Growing evidence identifies histamine as a key player in the immune responses, but the reports relating serum histamine to tolerance and to the risk of anaphylaxis are scarce and inconclusive.

This study aimed to investigate the association of serum histamine to the tolerant phenotype in beekeepers and to consider its value as a potential clinically relevant indicator.

Eight tolerant and 3 non-tolerant, medication-free beekeepers with a history of allergic reaction to HBV were evaluated though a questionnaire and subsequently underwent skin testing with purified HBV extract. Total immunoglobulin E (IgE), HBV specific IgE (sIgE) and IgG4 (sIgG4) levels were measured using ImmunoCAP®. Serum histamine was quantified fluorophotometrically.

Serum histamine levels were positively correlated with sIgG4 ($P < 0.05$) whereas neither variable was associated with total IgE or sIgE ($P > 0.05$). sIgG4 and histamine were negatively correlated with the time since the last bee sting. Histamine and sIgG4 levels followed comparable patterns, being higher in tolerant/recently stung individuals and lower in the least frequently stung subgroup. Total IgE and sIgE showed no significant relationship with any subgroup ($P > 0.05$).

This preliminary study associates, for the first time, serum histamine levels with sIgG4 and subsequently with HBV tolerance in beekeepers. Moreover, the findings provide the lead for further considering the potential regulatory role of histamine in immune responses and for the exploitation of serum histamine quantification as an indicator that could facilitate optimal allergic disease management.

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CURCUMIN ANALOGUES DIFFERENTIALLY MODULATE PRIMARY HUMAN BASOPHIL FUNCTION

B.F. Gibbs

Curcumin, a yellow pigment found in turmeric spice, has recently been shown to display therapeutic potential for the treatment of allergic diseases alongside its more established use as an anti-cancer agent. However, its cellular targets are not well defined and its bioavailability is low, which limits its wider therapeutic use. The aim of the present study was to assess whether curcumin affects basophil histamine release caused by IgE-dependent and—independent activation and to examine the inhibitory effects of curcumin analogues with known increased bioavailability. Human basophils were obtained from buffy coats purchased from the UK National Health Service following ethical approval (REC12/WM/0319). Basophils were enriched by Ficoll-density centrifugation and histamine releases were determined spectrofluorometrically after 30 min stimulation. Curcumin strikingly inhibited IgE-dependent histamine release from basophils by over 80 % at 30 μM and was even more efficacious at blocking fMLP-mediated histamine release, albeit being slightly less potent. The curcumin analogue (3E,5E)-3,5-bis[(2-fluorophenyl)methylene]-4-piperidinone (EF-24) displayed similar potency to curcumin as an inhibitor of IgE-dependent histamine release but was more efficacious and less toxic compared to curcumin (where toxicity was observed above 0.1 mM). Significant inhibitory actions were observed with both compounds at or above 1 μM. In contrast, other analogues, including coumaric acid, ferulic acid and caffeic acid only significantly inhibited histamine release at concentrations above 1 mM. These results demonstrate that the anti-allergic properties of curcumin are likely substantially to involve the attenuation of the release of histamine and other mediators from allergic effector cells. They also show that the more bioavailable analogue of curcumin, EF-24, retains these striking

inhibitory effects which highlights the therapeutic potential of this agent for the treatment of allergic diseases.

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IN THE SEARCH FOR NON DESCRIBED OLIVE TREE ALLERGENS BY TRANSCRIPTOME-MINING

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The olive tree (*Olea europaea* L.) is an important crop plant in Spain and the Mediterranean basin, its pollen representing an important source of allergens. Although some transcriptomes have been obtained from vegetative tissues, the peculiarity of reproductive tissues in terms of gene expression deserves a dedicated study not only for biological reasons but also in the search for allergens. For this purpose, Sanger sequences and Roche/454 reads were obtained from pollen at different developmental stages. After pre-processing, sequences were assembled, and annotated using a complex workflow, including the corresponding orthologues in *Arabidopsis thaliana* from TAIR and RefSeq databases. Using AutoFlow, an automated workflow has been constructed to import the annotated pollen transcriptome (27,823 transcripts) into a freely-accessible, web-browseable database called ReprOlive (http://reprolive.eez.csic.es/olivodb). Functional annotations of such transcriptomes are offering the possibility of exploring the presence of new variants of already described allergens in the olive pollen. In this respect, distinctive variants of the olive pollen allergen Ole e 5 have been identified and pollen NADPH oxidase homologs involved in allergy inflammation have been identified and analysed (unpublished work). Furthermore, this annotated transcriptome also allows the assessment of the putative occurrence of not yet described allergens, which might account for the complex allergogram of the pollen of this species. Hence, a massive and strict BLAST comparison was carried out between ReprOlive pollen transcripts and UniProt allergens (including those belonging to the specific database ALLERGOME, http://www.allergome.org). A list of putative new allergens has been obtained and the identification and analysis of possible antigenic regions in these proteins is currently underway.

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EFFECT OF H1-ANTIHISTAMINES ON HISTAMINE H_1 RECEPTOR GENE EXPRESSION IN THE NASAL MUCOSA OF PATIENTS WITH POLLINOSIS INDUCED BYARTIFICIAL EXPOSURE TO CEDAR POLLEN

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Stimulation of histamine H_1 receptors results in their up-regulation with a proliferation of receptor signalling. Elevation of histamine H_1 receptor mRNA has been observed in rat models of allergic rhinitis and treatment with H_1-antihistamines reduce gene expression. Thus, the H_1 receptor is thought to act as an allergic rhinitis sensitive gene.

The effect of antihistamines were examined on histamine H_1 receptor mRNA in the nasal mucosa of pollinosis patients induced by artificial cedar pollen exposure. Symptom provocation was induced by artificial cedar pollen exposure using an environmental exposure unit. Histamine H_1 receptor mRNA was determined by real-time quantitative RT-PCR.

Artificial pollen exposure provoked nasal symptoms and elevation of histamine H_1 receptor mRNA expressions in the nasal mucosa of some pollinosis patients. Prophylactic antihistamine treatment improved symptoms and also suppressed the elevation of H_1 receptor mRNA.

The histamine H_1 receptor gene may play a role as a disease sensitive gene. Improvement of symptoms in pollinosis as well as suppression of H_1 receptor gene expression can be accomplished by use of H_1 antihistamines.

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HISTAMINE MEDIATED REGULATION OF HUMAN MYELOID DENDRITIC CELL CYTOSKELETON AND FUNCTION

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Histamine receptors ($H_{1\text{-}4}R$) are differentially expressed in different cell types of the immune system. Among them, myeloid dendritic cells (DCs) were shown to be

functionally modulated through H_1R, H_2R and H_4R activation. HA shapes DC cytokine (CK) profile promoting Th2 polarization. Polarizing CKs production may be modulated by cytoskeleton modifications, hence we investigated the effects of HA on cytoskeleton organization on human monocyte derived DCs. To this aim, CD14+ monocytes were isolated from human buffy-coat of 5 healthy donors and differentiated in vitro towards DCs with GM-CSF and IL-4 for 7 days; at day 6 (D6), DCs were treated with HA (10 μM), the H_1R antagonist pyrilamine (10 μM), the H_2R antagonist zolantidine (10 μM), the H_4R antagonist JNJ7777120 (10 μM), or various combinations of HA with antagonists. At D6 DCs were stimulated with LPS for 24 h, whereas controls received vehicle. At D7 DCs were analyzed for actin distribution by confocal microscopy; phenotype by flow cytometry; CK production by ELISA. At different time points during culture, DCs were analyzed for $H_{1\text{-}4}R$ expression by real time PCR. Confocal microscopy analysis revealed that HA treated DCs had a more immature morphology compared to controls; in >50 % of DCs f-actin and CD11c co-localized on cell membrane of podosome-like structures, typical features of immature DCs. According to this observation, Rac-1 and PAK-1, molecules involved in cytoskeleton regulation, had a different distribution in HA treated DCs compared to controls ($p < 0.05$, Student *t* test). Moreover, HA treatment significantly reduced IL-12p70 production ($p < 0.05$, ANOVA with Bonferroni's post hoc test)). These effects were partially blocked by zolantidine, but were unaffected by JNJ7777120 or pyrilamine. Of note, HA did not affect the expression of typical DC maturation and activation markers CD80, CD86, HLA-DR, CD83. Our data demonstrate for the first time the role of HA in modifying DCs cytoskeleton organization towards a decreased production of IL-12p70.

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A ROLE FOR ANTIZYME INHIBITOR 2 IN THE BIOSYNTHESIS AND CONTENT OF HISTAMINE AND SEROTONIN IN MOUSE MAST CELLS

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Polyamines (putrescine, spermidine and spermine; PAs) are required for the survival of the majority of living cells. Antizymes and antizyme inhibitors are key regulatory proteins of PA levels by affecting ornithine decarboxylase (the rate-limiting biosynthetic enzyme) and PA uptake. In addition to PAs, mast cells (MCs) synthesize and store in their granules histamine (Hi) and serotonin (5-HT), which are critical for their function. Previously, we have performed several studies regarding the interplay between the metabolisms of PAs, Hi and 5-HT in this cell type. Our results showed that PAs affect Hi synthesis during early stages of IL-3-induced bone marrow cell differentiation into bone marrow derived MCs (BMMCs) and demonstrated that PAs are present in MC secretory granules. Also, they are important for granule homeostasis, including Hi storage and 5-HT levels. A few years ago, a novel antizyme inhibitor (AZIN2) was described. In contrast to AZIN1, AZIN2 expression is restricted to a few tissues and cell types including brain, testis and MCs. In MCs, it was recently described that AZIN2 could act as a local regulator of PA biosynthesis in association with the 5-HT granule content and release. Our aim here is to gain further insight into the role of AZIN2 in the biosynthesis, storage and release of both Hi and 5-HT. In this study, we have generated BMMCs from both wild-type and transgenic mice with severe Azin2 hypomorphism and have analyzed the content of PAs, Hi and 5-HT, and some elements of their metabolism. Both spermine and 5-HT levels were reduced in Azin2 hypomorphic BMMCs compared with wild-type controls (7.9 ± 0.5 vs 9.6 ± 0.5 and 47.2 ± 13 vs 80.8 ± 13.3 pmol amine/μg protein, respectively; $n = 3$, $P < 0.02$), while the amount of Hi showed a clear trend to increase (111.2 ± 39.1 vs 56.3 ± 17.9 pmol Hi/μg protein; $n = 4$, $P = 0.09$). Accordingly, the level of tryptophan hydroxylase 1 (the key enzyme for 5-HT biosynthesis) was reduced in Azin2 hypomorphic BMMCs as judged by immunoblot ($n = 3$), whereas the amount of enzymatic activity of histidine decarboxylase (the enzyme responsible for histamine biosynthesis) showed a trend to increase (0.37 ± 0.1 vs 0.16 ± 0.02 pmol CO_2/h/μg protein; $n = 3$, $p = 0.12$). Altogether, our results show that AZIN2 has an important role in the regulation of Hi and 5-HT biosynthesis and storage in MCs.

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TH9 CELLS EXPRESS HISTAMINE RECEPTOR SUBTYPES AND RESPOND TO HISTAMINE

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Th9 cells, characterized by a high production of IL-9, are the most recently defined subset of $CD4^+$ T-helper cells. They develop from naive CD4+ T-helper cells in the

presence of IL-4 and TGFβ and are involved in autoimmune diseases and allergic inflammation. It was shown that Th9 percentage and IL-9 expression levels of atopic dermatitis patients were increased compared to healthy controls. As histamine is also upregulated in lesions of inflammatory skin diseases, we decided to investigate the role of histamine and its receptors on differentiation and regulation of Th9 cells.

Therefore, naïve CD4+ T- cells were isolated from PBMCs and incubated for 5 days in IAB medium containing IL-2, aCD3 and aCD28. These so called Th0 cells were further stimulated with IL-2, aCD3, aCD28, IL-4 and TGFβ for differentiation into Th9 cells. As proof of a successful differentiation, IL-9 production was measured at mRNA as well as protein level. Next, the histamine receptor expression levels were investigated. The expression of H_1R, H_2R and H_4R was significantly upregulated on Th9 cells compared to the expression on Th0 cells. Additionally, incubation with histamine during differentiation showed an increase in IL-9 secretion compared to the normal differentiation protocol. Further studies with receptor specific ligands are ongoing.

Taken together, our study demonstrates a functional effect of histamine on Th9 cells.

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RECEPTIVE MUSIC THERAPY (RMT), FOOD ALLERGY AND HISTAMINE

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Receptive Music Therapy (rMT) is used to support psychological, physical and mental health. The question arises as to whether music can also influence food allergy and histamine responses.

A group of 12 volunteers (students 19–43 years, six (2 males, 4 females) with increased plasma IgE, and six matched controls with normal total IgE) were tested by using a simple experimental design. The tested persons were exposed to "good feeling" music while eating "adverse" (individual aversion to special food like uncooked egg and fish) test foods. Anamneses and interview reports served as a basic reference assessment. Blood was drawn from the arm vein, saliva from the mouth before and not later than 5 min after challenges. Plasma and saliva histamine and IgE were measured using ELISA (Beckman-Coulter) and histamine was evaluated using fluorimetry after HPLC separation.

The majority of the tested volunteers showed significant responses to eating adverse diets ($p < 0.05$, $n = 12$, Student's *t* test). Pulse rates were elevated from 80 ± 6 to 94 ± 8 min^{-1} and plasma histamine levels increased from 0.3 ± 0.2 to 0.85 ± 0.3 ng/ml during eating. No significant differences in plasma histamine concentrations could be detected between the atopic (A) (at least 1 food allergen and specific IgE RAST >1) and the non-atopic (NA) groups ($p > 0.05$) during rMT. It could be shown that saliva histamine ranged in the atopic patients from 0.05 ng/ml up to 4 ng/ml. This was significantly suppressed during rMT (A: suppression $= 59.5 \pm 23.5$ %, $p < 0.01$, 4 test persons). No suppressive responses were observed in non-allergic matched controls (NA: saliva histamine range 0.5 up to 11.3 ng/ml). As described previously, pulse rates were significantly reduced in all experiments after "good feeling" music exposure. This observation was also true even when the pulse rate was elevated after eating "adverse food" in both the A and in the NA-groups. It can be concluded that histamine in saliva is a suitable tool for measurements of the physiological effects of rMT.

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CROSS-TALK BETWEEN SERUM HISTAMINE LEVELS AND SKIN REACTIVITY TO HISTAMINE IN ALLERGIC SUBJECTS

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Skin prick tests (SPTs) are commonly used to determine IgE sensitization in allergic individuals. Histamine typically serves as a positive control in SPTs and provides a measure of non-specific skin reactivity. Yet, many factors, including age, gender, menstrual cycle and drug intake may influence the skin response to histamine. The aim of the study was to explore any association between serum histamine levels and the histamine-induced wheal and flare response in SPTs.

Eleven medication-free subjects allergic to honey bee venom (HBV) underwent SPTs with purified HBV. Normal saline and 10 mg/ml histamine·2HCl were used as the negative and positive controls, respectively. Intradermal (ID) skin tests were also performed using purified HBV extract. A mean wheal diameter of ≥3 mm relative to the negative control was considered as a positive SPT or ID test and revealed sensitization to HBV. Serum levels of total immunoglobulin E (IgE) and HBV specific IgE (sIgE) were determined using ImmunoCAP®. Serum histamine levels were quantified fluorophotometrically.

Serum histamine levels showed a significant positive correlation with the diameter of the wheal observed with histamine (Spearman's $\rho = 0.69$, $P < 0.05$) but not with that obtained with HBV (Spearman's $\rho = -0.185$, $P > 0.05$) in skin tests. The wheal sizes obtained with histamine and HBV were unrelated to each other (Spearman's $\rho = 0.048$, $P > 0.05$). Moreover, the skin responses provoked by either histamine or HBV showed no significant relationship to the levels of either the total IgE or sIgE to HBV ($P > 0.05$, Spearman's test).

The observed positive correlation between skin reactivity to histamine, but not to HBV, and serum levels of histamine, but not total IgE or sIgE, argues for a cross-talk between dermal and serum histamine-mediated mechanisms on diverse effector cells and/or pathways. The potential (patho)physiological significance and the clinical relevance of these findings need to be carefully considered.

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TH1/TH2 CYTOKINE PROFILES IN MONO—AND POLYSENSITIZED ALLERGIC PATIENTS

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Hyperproduction of Th2 type cytokines (IL-4, IL-5, and IL-13) is thought to be the key factors in the development of IgE-mediated allergic diseases. Most patients with allergic rhinitis, asthma, and atopic dermatitis have multiple sensitivity to different allergens from different sources. Therefore, we addressed the question: are cytokine profiles/cytokine activities identical in patients with mono—and polyvalent allergies or not? The objective of this investigation was to study Th1/Th2-like cytokine profiles in sera of patients with monovalent pollen allergy and polyvalent allergy with combined hypersensitivity to pollen allergens and house dust mites.

The concentration of Th1/Th2 cytokines were investigated, in the sera of 13 patients with allergic rhinitis, sensitive to ragweed pollen, and 14 patients with polyvalent allergy to ragweed pollen and house dust mites, by Multiplex assays using Luminex xMAP technology. The diagnosis of allergies was confirmed by anamnesis, prick tests and serological investigations of specific IgE antibodies which were assayed by ELISA using recombinant allergens to ragweed pollen (Amb1, Dep p1).

In the group with polyvalent allergy, we observed increased concentrations of IFNγ and IL-18 by 1.5 and 1.3-fold, correspondingly. In the same group, the concentration of IL-4 was double than that in patients with monovalent pollen sensitivity. In the polyvalent group of patients the level of IL-5 was detectable in comparison to the monovalent sensitivity group of patients in which the concentration of this cytokine was below detectability. Interestingly, IL-6 concentrations were increased in patients with pollen allergy in comparison to polysensitized allergic patients.

This study reveals differences in cytokine profiles between mono- and poly-sensitized allergic patients. Patients with more than one sensitization had increased levels of IgE regulatory cytokines (Th1 as well as Th2-type). This effect may be due to the non-specific activation of different subtypes of T cells induced by several allergens from different sources.

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ADHESION OF HUMAN EOSINOPHILS TO ENDOTHELIAL CELLS—EFFECTS OF SELECTIVE HISTAMINE RECEPTORS ANTAGONISTS ON THE ADHESION PROPERTIES OF EOSINOPHILS

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Histamine is an organic amine which has broad physiological actions. This is due to the fact that histamine has a unique ability to act through four types of G-protein-coupled proteins, known as histamine H_1, H_2, H_3 and H_4 receptors. It has been demonstrated that histamine plays an important immunomodulatory role, mainly in immune cell chemotaxis through activation of histamine H_4 receptors. To date there are limited data concerning the effects of histamine on immune cell adhesion to endothelial cells, which is an important stage in immune cell trafficking. The aim of the present study was to estimate the role of histamine and selective histamine receptor antagonists on the process of eosinophil adhesion to endothelial cells. Human eosinophils were isolated from fresh human blood by Ficoll-Paque density gradient separation, followed by negative immunomagnetic cell sorting. Viability and functionality of the isolated cells were assessed by trypan blue staining, MTS assay, determining levels of apoptosis and by eosinophil peroxydase (EPO) release assay. The effects of histamine on eosinophil adhesion to endothelial cells were evaluated during co-culture of eosinophils with human Ea.hy.926 endothelial cell lines under static conditions. The role of histamine receptors in controlling cellular adhesion was tested using an appropriate set of antagonists. Pure

eosinophil populations were isolated from human blood and characterized by high viability and functionality. Stimulation of eosinophils with histamine and selective histamine receptors antagonists resulted in different adhesion efficiencies to endothelial cells. These results highlight an important role of histamine in the adhesion of eosinophils to endothelial cells.

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

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Mast cell mediators play a critical role in many type 1 hypersensitivity reactions including allergic rhinitis, conjunctivitis and asthma, and in the progression of many different cancers. Thus, there is still an unmet need to develop new agents which prevent mast cell mediator release or which inhibit the actions of such mediators once released into the environment of the cell. The aim of this work was to conduct an assessment of the potential anti-allergic activity of a novel series of tetralone and benzosuberone derivatives. The primary steps in their synthesis involved the dialkylation of the parent benzocycloalkanone with a variety of allylic and benzylic halides. These intermediates were then reduced to their corresponding alcohols using either sodium borohydride or lithium aluminium hydride. In some examples, these alcohols were further modified with a view to improving their solubility by the attachment of a variety of water-solublising amino and carboxy groups. Their mast cell stabilising properties were evaluated using percoll-purified rat peritoneal mast cells when compound 48/80 and calcium ionophore A231867 were used to induce degranulation. *In vitro* investigation of the mast cell stabilising activity revealed that optimal activity appeared to reside in derivatives bearing benzylic groups, while ring expansion of the hydroaromatic core is permissible without loss of activity in vitro. The key compound for this study was TZ2.05.

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ANTI-INFLAMMATORY AND ANTI-FIBROTIC EFFECTS OF A SELECTIVE PARP-1 INHIBITOR IN A MURINE MODEL OF BLEOMYCIN-INDUCED LUNG FIBROSIS

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Pulmonary fibrosis is characterized by inflammation, excessive collagen deposition and abnormal remodeling of lung parenchyma, resulting in airway stiffening and thickening of the air-blood membrane. Effective therapies are not available and novel therapeutic strategies are urgently required, including molecular targeting of specific signaling pathways activated during fibrotic processes.

Poly(ADP-ribose)polymerase (PARP) is a family of enzymes, involved in DNA repair and apoptosis. PARP1, the most abundant enzyme, modulates the expression of inflammatory genes and can be activated by reactive oxygen species. Protective effects of PARP inhibition in asthma models have been identified and our previous study showed that PARP1 inhibition decreases tissue damage induced by oxidative stress.

We investigated the effects of a selective PARP1 inhibitor, HYDAMTIQ (hydroxyl-dimethylaminomethyl-thieno[2,3c] isoquinolin-5(4H)-one), in a murine model of bleomycin-induced lung fibrosis. Ten C57BL/6 mice were treated with saline and 60 with bleomycin (0.05 IU), both intra-tracheally. Fifteen mice per group received HYDAMTIQ (1-3-10 mg/kg b.wt) or vehicle i.p. for 21 days. Airway resistance to inflation, an index of lung stiffness, was assayed and lung tissues were processed for inflammation, oxidative stress and fibrosis markers.

Our results indicate that HYDAMTIQ exerts an anti-inflammatory and anti-fibrotic effect, as shown by the reduction of pro-inflammatory (TNFα, 5.69 ± 0.06 ng/μg of total protein in vehicle vs 0.48 ± 0.11 ng/μg of total protein in 10 mg/kg treated animals, $p < 0.005$; IL1β, 13.85 ± 1.26 pg/μg of total protein in vehicle *vs* 0.89 ± 0.03 pg/μg of total protein in 10 mg/kg treated animals, $p < 0.05$) and pro-fibrotic (TGFβ1, 164.6 ± 7.1 pg/μg of total protein in vehicle vs 14.7 ± 0.7 pg/μg of total protein in 10 mg/kg treated animals; $p < 0{,}005$) cytokine production. Moreover HYDAMTIQ reduces collagen deposition, modulating the activation of p-Smad3 signaling pathway. It decreases oxidative stress, reducing 8OHdG (0.264 ± 0.111 ng/μg of DNA in vehicle vs 0.074 ± 0.029 ng/μg of DNA in 10 mg/kg treated animals; $p < 0.05$), and it seems to exert a protective effect, reducing the expression of iNOS and COX2.

These results suggest that PARP inhibitors could be a promising approach to evaluate the possible anti-fibrotic potential of these molecules. We hypothesize that selective PARP1 inhibitors can reduce signs and symptoms of lung fibrosis and control post-inflammatory bronchial remodeling and hyper-responsiveness.

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Neurological aspects of histamine

ANALYSIS OF HISTAMINE *N*-METHYLTRANSFERASE DEFICIENT MICE

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Histamine clearance is essential for maintaining the homeostasis of histaminergic neuronal activities. Although it has been elucidated that brain histamine was inactivated by histamine *N*-methyltransferase (HNMT), the importance of HNMT in vivo was remained unclear. In the present study, we generated and investigated the HNMT knockout mice (KO) to clear the importance of HNMT in vivo.

First, we prepared KO by inserting LacZ gene into HNMT gene. LacZ reporter assay showed that HNMT strongly expressed in cortex, ambiguous nucleus and medial vestibular nucleus. Histamine content in the brain lysate in KO was 8-times as much as that in WT ($n = 8$, $p < 0.05$). In addition, the extracellular histamine in the hypothalamic area was significantly increased, indicating that HNMT was essential for brain histamine clearance. The anxiety-like behaviours, working memory and social interaction were not changed. On the other hand, the movement time, distance and speed of KO were significantly decreased compared to wild type (WT) in open field test. The locomotor activity of KO in home cages was decreased in the dark period with prolonged immobility time ($n = 10$, $p < 0.05$). In addition, the number of wounded mice was significantly higher than WT in home cages, and the increase of the aggressive behaviours could be checked by resident-intruder test. The isolation stress-induced disruption of prepulse inhibition was not observed in KO. These results indicated HNMT was involved in locomotor activity, aggressive behaviour and isolation stress.

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HISTAMINE AND OREXIN: FROM WAKE TO SLEEP

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More than a decade ago it was demonstrated that canine narcolepsy is caused by a mutation in the OX-2 receptor gene. This discovery, taken together with the observation that OX-2 immunoreactive nerve terminals are exclusively expressed in histaminergic neurons within the tuberomamillary nucleus of the lateral hypothalamus, prompted the evaluation of histamine H_3 antagonists in a canine model of narcolepsy. Thus it was shown that H_3 antagonists significantly reduced cataplexy in narcoleptic Dobermans. Having exploited the role of OX-2 receptors in a debilitating sleep disorder we turned our attention to establishing the utility for selective OX-2 receptor antagonists for the treatment of insomnia. The discovery, preclinical and clinical evaluation of a selective OX-2 antagonist was presented.

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HISTAMINE IN THE BASOLATERAL AMYGDALA CONTRIBUTES TO OLEOYLETHANOLAMIDE-INDUCED PROCOGNITIVE EFFECTS

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The ability to remember contexts associated with food availability and palatability provides a clear adaptive advantage to animals foraging. In this context, it was demonstrated that the satiety factor oleoylethanolamide (OEA) facilitates memory consolidation in emotional and spatial memory. We recently observed that neuronal histamine (HA) participates to OEA hypophagic effects, and large evidence indicate that the HA system is involved in mnemonic processes. Thus, we investigated if brain HA is also required for OEA-induced promnesic effects. Wistar rats (280-300 g) were submitted to the contextual fear conditioned paradigm and immediately after received a single OEA injection (10 mg/kg, i.p.). Memory was assessed as 'freezing' (total absence of movements) time 72 h after injections. OEA-treated animals showed a longer freezing time (255.3 ± 48.5 s; $n = 11$, $P < 0.05$ unpaired t-test) as compared to saline-treated animals (176.6 ± 34.4 s; $n = 8$). Pre-treatment with the histamine biosynthesis inhibitor alpha-fluoromethylhistidine (5 μg, i.c.v.) prevented such effect (150.7 ± 61.3 s, $n = 9$). Next we investigated HA release from the brain of freely moving rats, using the microdialysis. After OEA i.p. injection, at the same dosage used in the behavioural paradigm, we observed a fast and transient increase (up to 120 %, $P < 0.05$ ANOVA and Bonferroni's test) of HA release only from the basolateral amygdala (BLA), a histaminergic projection area critically involved in the modulation of aversive memories (basal HA release, 66.8 ± 23.1 fmol/15 min, $n = 5$). OEA did not affect HA release from the hypothalamic ventromedial and paraventricular nuclei that mediate OEA hypophagic effects, whereas it decreased HA release

from the prefrontal cortex and nucleus accumbens (n = 4–5 per group). Our results suggest the requirement of neuronal histamine in the BLA for OEA to induce promnesic effects in the fear conditioning paradigm.

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A LOCAL CIRCADIAN CLOCK IN HISTAMINERGIC NEURONS INFLUENCES SLEEP ARCHITECTURE

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Circadian clocks allow prediction of environmental events in the 24 h cycle. In terms of a sleep drive, two factors are believed to produce sleep: circadian and homeostatic. The master circadian clock is in the suprachaismatic nucleus, but clock genes are expressed throughout the brain and indeed, most body tissues. We tested if a putative local clock in mouse histaminergic neurons in the tuberomamillary nucleus in the hypothalamus regulated the sleep-wake cycle. By real-time PCR, we found that histidine decarboxylase (*hdc*) gene expression varied with time of day about 1.5-fold (unpaired two-tailed, t test, $p < 0.05$). By crossing *HDC-Cre* mice with $Bmal1^{lox/lox}$ mice, we selectively deleted the *Bmal1*clock gene from histaminergic cells and found this removed this variation in *hdc* gene expression, producing higher HDC expression and brain histamine levels during the day (two-way ANOVA and post hoc Bonferroni, $p < 0.05$). Using EEG recordings from the mice, we found that the consequences included more fragmented sleep, prolonged wake at night, shallower sleep depth, increased NREM to REM sleep transitions, and hindered recovery sleep after sleep deprivation. The mice also had impaired memory, as assessed by novel object recognition (one-way ANOVA and post hoc Bonferroni, $p < 0.01$). In some ways the mice lacking BMAL1 in their histaminergic cells had the opposite phenotype to *hdc* knockout mice, as might be expected given that *hdc* transcript levels were on average higher in the BMAL1 knockouts. We propose that the local BMAL1-dependent clock mechanism suppresses daytime histaminergic tone and helps facilitate appropriately timed intervals of sleep and wake synchronised to the animal's overall circadian behavior. Such local clocks link the two types of sleep drive, circadian and homeostatic together. Our results also suggest that histaminergic synthesis in other cell types, such as mast cells, could also be influenced by cell-intrinsic local circadian clocks.

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ON THE ROLE OF HISTAMINE RECEPTORS IN REGULATION OF CIRCADIAN RHYTHMS

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Several lines of evidence suggest a regulatory role of histamine in circadian rhythms. The histidine decarboxylase knockout mice exhibit abnormal circadian rhythms of locomotor activity and expression of core "clock" genes—*BMAL1, Per2*—in striatum and cerebral cortex, and pharmacological inhibition of histidine decarboxylase in rats causes significant attenuation of the amplitude of rhythms of adrenocorticotropic hormone and corticosterone. Despite these observations, little is known about exact molecular mechanisms of the histaminergic regulation of circadian rhythms. Thus, the aim of this study was to examine whether or not histamine mediates its effects on circadian system through *Hrh1* or *Hrh3* receptors.

In order to assess free-running locomotor activity rhythms of $hrh1^{-/-}$ and $hrh3^{-/-}$ mice, the animals were housed in complete darkness for two weeks and their motor activities were video-recorded. We found that free-running activity rhythms of knockout mice were 23.71 ± 0.09 h ($p < 0.001$, χ^2-periodogram) which was indistinguishable from that of wildtype counterparts ($n = 24$, $p = 0.31$, Student's t-test). We further examined whether expression of circadian clock genes, *BMAL1* and *Per2*, was altered in knockout animals. The expression of both genes was assessed at ZT6 and ZT14 in cortex, striatum and suprachiasmatic nuclei by means of radioactive in situ hybridization. In these structures no significant differences between knockout and wild type animals were found ($p > 0.05$, maximum likelihood estimation, n = 6–9 per group).

We conclude that either the effect of $Hrh1^{-/-}$ and $Hrh3^{-/-}$ gene knockouts on circadian system was completely compensated during development or these receptors do not take part in regulation of the tested parameters.

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HISTAMINE MODULATES DOPAMINERGIC NEURONAL SURVIVAL BY BOOSTING MICROGLIAL ACTIVITY

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Histamine is an amine widely known as a peripheral inflammatory mediator and a neurotransmitter at the Central Nervous System (CNS). Recently, it has been suggested that histamine acts as an innate modulator of the microglial activity. Herein, we aimed to disclose the role of histamine in microglial phagocytic activity and reactive oxygen species (ROS) production; and to explore the consequences of histamine-induced microglia inflammation in dopaminergic neuronal survival. First, we found that histamine triggers microglial phagocytosis (about 2.5-fold increase as compared to control, $n = 5$-15, $P < 0.001$) via H_1R activation and ROS production (about 1.4-fold increase as compared to control, $n = 3$–16, $P < 0.001$) via H_1R and H_4R activation. These effects were accompanied by the rearrangement of microglial cytoskeleton monitored through phalloidin and acetylated α-tubulin immunostaining. By using apocynin, a NADPH oxidase inhibitor, and Nox1 knock-out mice, we found that the Rac1/Nox1 signaling pathway is involved in both phagocytosis and ROS production induced by histamine. Interestingly, both apocynin and annexin V (used as inhibitor of phosphatydilserine-induced phagocytosis) fully abolished the dopaminergic neurotoxicity induced by the injection of histamine in the substantia nigra in vivo (about 30 % decrease of TH + cells as compared with saline-treated mice, $n = 3$–7 mice, $P < 0.001$). Moreover, the co-localization between Cd11b+, TH+ and PtdSer residues upon histamine treatment in vivo, suggests that stressed dopaminergic neurons expressing PtdSer residues on their membrane could became a target for microglial phagocytosis and subsequent cell death. Overall, our results highlight the relevance of histamine in the modulation of microglial activity that ultimately may interfere with neuronal survival in the context of Parkinson's disease and, eventually, other neurodegenerative diseases which are accompanied by microglia-derived inflammation. Importantly, our results also open promising new perspectives for the therapeutic use of H_1R or H_4R antagonists to treat or ameliorate neurodegenerative processes associated with neuroinflammation induced by histamine.

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LACK OF cAMP RESPONSE ELEMENT-BINDING PROTEIN (CREB) ACTIVATION PARALLELS ALPHA-FLUOROMETHYLHISTIDINE (a-FMHis)-INDUCED IMPAIRMENT OF LONG-TERM MEMORY OF STEP-DOWN INHIBITORY AVOIDANCE (IA)

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We reported last year that histamine depletion by i.c.v. administration of alpha-fluoromethylhistidine (a-FMH, 5 μg/5 μl, a suicide inhibitor of histidine decarboxylase) impairs long- but not short-term memory of rats trained in the inhibitory avoidance (IA) paradigm. To find a possible mechanism of this effect, we studied IA-induced CREB phosphorylation in amygdala and dorsal hippocampus (DH) of histamine depleted rats and controls, as CREB activation in the DH is crucial in the formation of long-term memory. We hypothesize that for a short period after training, the DH acts in concert with the BLA to contribute emotional values to aversive events. pCREB levels were assessed at 10 min and 5 h post-training in the amygdala and DH of rats given icv saline or a-FMH 24 h before training. pCREB levels were measured by Western blots analysis. Controls received saline/no foot-shock after stepping down the platform. Rats receiving saline and footshock without stepping down were also investigated (untrained/footshock). At 10 min post-training, pCREB levels in controls and untrained/footshock animals were not different in either brain areas. Conversely, both amygdala and DH of rats given saline or a-FMH displayed a significant increase of pCREB levels (ANOVA and Bonferroni's MCT, amygdala: $F_{3,15} = 11.64$; $P < 0.0007$; DH: $F_{3,15} = 7.3$; $P < 0.004$) as compared to controls suggesting that histamine is not involved in the activation of CREB immediately after training. At 5 h post-training, no difference of pCREB levels was found in the amygdala of all groups. In the DH, however, rats treated with saline but not those with a-FMHis showed a significant increase of pCREB levels as compared to controls ($F_{2,19} = 7.498$; $P < 0.004$). Therefore, histamine neurotransmission in the DH seems to be crucial for aversive long-term memory formation and also for hippocampal CREB phosphorylation that accompanies this training.

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ZEBRAFISH BRAIN HISTAMINE SYSTEM ANALYSIS WITH CRISPR/CAS9 GENOME EDITING METHODS

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Histamine is an important transmitter in the brain, and zebrafish is a useful model to study its role in behaviour and disease models. However, no reliable zebrafish mutants have been available to analyse the significance of relevant genes. The zebrafish genome contains one *hdc, hrh1* and *hrh2* genes, one characterized *hrh3* gene and two additional *hrh3*-like genes. We have applied clustered, regularly interspaced, short palindromic repeats (CRISPR) and the CRISPR-associated system (CRISPR/Cas system) to produce and characterize fish mutants. Guide RNAs (gRNAs) were designed to target exon 2 of *hrh3*, exon1of *hrh3B* and *hrh3C*. The gRNA/Cas 9 mRNA injected embryos were raised to adulthood (P0) and their offspring was screened (F1). Of the twenty-four *hrh3* F1 clutches analysed, we identified eleven that carried nucleotide deletions in one allele of *hrh3* locus, which caused frameshift mutations resulting in truncated proteins. The heterozygous fish were morphologically normal, did not have obvious gross phenotype, and showed normal spontaneous behaviour and fertility. *hrh3B* and *hrh3C* mutant screening is in progress.

The results indicate that it is possible to generate targeted zebrafish mutants to fully assess the significance of histamine and histamine receptors in zebrafish using multidisciplinary phenotyping methods, and to reveal the role of histamine in brain functions.

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CHARACTERIZATION OF HDC KO MICE: STEREOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Histidine decarboxylase knock-out (HDC KO) mice lack the enzyme responsible for histamine production. These mice show numerous behavioral phenotypes including attenuated feeding behavior, altered sleep-wake cycle and display weaker stimulatory response to acute ethanol. Furthermore, recent data connects HDC deficiency with familial Gilles de la Tourette syndrome (GTS).

Human in vivo and postmortem studies have revealed that GTS patients have reduced volume of the caudate nucleus and cortical thinning, while the volume of the putamen is increased and the number of striatal parvalbumin and cholinergic interneurons is decreased. We hypothesized that related abnormalities should be found in the brains of HDC KO mice, if lack of histamine is responsible for the phenotype.

We used unbiased stereology for estimating the number of parvalbumin, calretinin, nitric oxide synthase and choline acetyltransferase positive interneurons in cortex and striatum of HDC deficient mice. Volumes of the striatum and cortex were estimated by unbiased stereological method, Cavalieri estimator. The results of stereological and volumetric measurements showed no significant differences between control and HDC KO mice. We also examined cortical morphology and layering with Nissl staining and immunohistochemical markers (Cux1, FoxP2, and VGlut2) and found no abnormalities in early postnatal (P9-P10) and adult HDC KO mice.

Our results indicate that HDC deficiency in mice as such does not lead to the morphological and cytological phenotypes commonly seen in humans with GTS.

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H_1-KNOCKED OUT MOUSE HAS HIGH SENSITIVITY TO ISOFLURANE-INDUCED ANESTHESIA

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Inhaled general anaesthetics induce loss of consciousness (LOC) via the modulation of arousal/sleep pathways in central nervous system. Histamine and histamine H_1 receptor (H_1) in brain have important functions in the regulation of arousal/sleep cycle, but it remains largely unknown about the involvement of histaminergic neurons in LOC induced by inhaled anaesthetics. Therefore we have attempted to elucidate the role of H_1 in inhaled isoflurane-induced anaesthesia. Initially we examined the effect of isoflurane (an inhaled general anaesthetic) on histamine release in the central nervous system. We performed in vivo microdialysis to measure extracellular histamine concentration in mouse hypothalamic area under various concentrations of isoflurane. Histamine was increased 1.2 times higher at low concentrations of isoflurane, while it decreased below the baseline level at high concentrations ($n = 3, p < 0.05$). We also found that H_1-knocked out mice (H_1KO) had shorter induction time (134.9 ± 19.87 vs 211.5 ± 9.231 s, $n = 7$, $p < 0.05$), lower EC_{50} to loss of righting reflex (0.7893 vs 1.197 %, $n = 10$) and longer

emergence time (113 ± 18.90 vs 35.4 ± 20.43 s, n = 7, $p < 0.05$) to isoflurane-induced anaesthesia. On examining the behaviours of H_1KO under isoflurane-induced anaesthesia we found that they had longer escape latency from hotplate-induced nociception compared to wild type (17.1 ± 6.99 vs 4.85 ± 1.71 s, n = 5, $p < 0.05$). Therefore these results indicate neuronal histamine and H_1 are involved in LOC induced by isoflurane.

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H_4R ANTAGONIST AND NITRIC OXIDE INHIBITORS: INTERACTIONS IN ANTINOCICEPTION

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Nitric oxide (NO) is involved in several physiological processes, inter alia in the nociception. In our previous study, we have reported that H_4R antagonist JNJ7777120 (J77) produced antinociception which can be potentiated by a non-specific nitric oxide synthases (NOS) inhibitor. In this study, our aim was to identify which NOS isoforms are responsible for observed interactions.

Male WAG rats received tested compounds intraperitoneally either alone or in combination. Pain threshold was measured versus mechanical (Randall-Selitto test) and thermal stimuli (Tail Flick test). Results are presented as % of antinociception. H_4R inverse agonist JNJ7777120 (J77) (20 mg/kg) exhibited clear antinociceptive effect both to mechanical and thermal stimuli (32 ± 7 and 42 ± 8 %, n = 6, respectively). Coadministration of non-specific NOS inhibitor L-No-Arg (10 mg/kg) increased antinociceptive effect (73 ± 9 and 98 ± 8 %, n = 6, $p < 0.01$, respectively). Coadministration with specific nNOS inhibitor -7-NI (1 mg/kg) weaken antinociceptive action of J77 vs. mechanical stimuli (9 ± 4 %, n = 6, $p < 0.01$), but increased vs thermal stimuli (61 ± 14 %, n = 6, $p < 0.01$), endothelial NOS inhibitor—L-NIO (1 mg/kg) potentiated antinociceptive action of J77 vs. mechanical stimuli (63 ± 16 %, n = 6, $p < 0.01$); inducible NOS inhibitor—L-NIL (1 mg/kg) coadministration decreased antinociceptive action of J77 vs both types of stimuli (10 ± 1 and 11 ± 2 %, n = 6, $p < 0.01$, respectively).

Differential effects of specific NOS inhibitors require further studies. Because various painful stimuli are experienced by different nociceptors, conducted by various fibers and can be differentially modified by complex regulatory systems. It is probable that H_4R may be differentially expressed on these structures.

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THE ROLE OF PRESENILIN1 IN NEUROGENESIS IN ZEBRAFISH, DANIO RERIO

M. Sundvik, P. Panula

Histaminergic neurons play a major role in an array of different behaviors executed by the vertebrate brain. So far, the sole regulator of the development of the histaminergic neurons was recently shown to be the Alzheimer's disease associated presenilin1 (psen1) gene. There are two presenilin genes, which both function as the catalytic subunit of γ-secretase. The γ-secretase has tens of different substrates of which Notch and amyloid precursor protein are best known. We have previously shown that the number of histamine neurons in psen1−/− fish is lower than in wild-type control animals during development and that the number of histamine neurons in the old psen1−/− fish is increased compared with control animals. We hypothesized that this could be due to either neurotransmsitter respecification or neurogenesis. Thus immunohistochemical analysis of markers associated with neurogenesis was undertaken. We studied sox2, pax6a and NeuroD expression in psen1−/− and control zebrafish at three days post fertilization which did not reveal any difference in expression (n = 5/group). Neither did we find a difference in neurogenesis of seven days post fertilization old zebrafish larvae as incorporation of 5-bromo-2′-deoxyuridine was not altered between the two different genotypes (n = 16–18). Histaminergic neurons are located in the caudal hypothalamus, surrounding the posterior recess of the diencephalic ventricle. Immunohistochemical studies of sox2, pax6a, NeuroD, PCNA and ki67 in the old psen1−/− animals revealed an increase in the number of sox2 and pax6a immunoreactive neurons in the inferior lobe of the posterior hypothalamus when compared with control animals (n = 7/group). No difference in numbers of neuronal precursor cells was observed in the histaminergic caudal hypothalamus. These findings suggest that the increase in histaminergic neuron number observed in adult psen1−/− is due to neurotransmitter respecification rather than neurogenesis within the histaminergic caudal hypothalamus.

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Histamine H_4 receptor

REAL TIME QUANTIFICATION OF INTRACELLULAR HUMAN H_4R TRAFFICKING USING BRET

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Agonist-induced G protein-coupled receptor (GPCR) activation leads to desensitization and internalization of the receptor into the cell. Internalized GPCRs are either degraded or recycled to the cell surface.

In this study, internalization and intracellular trafficking of the human H_4R is investigated using specifically localized bioluminescence resonance energy transfer (BRET) sensors to plasma membrane, early endosomes, late endosomes and recycling endosomes. This allows us to follow the intracellular receptor trafficking in real time and will reveal more insights in the signaling and trafficking mechanisms of the human H_4R.

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PRODUCTION OF M2 MACROPHAGE-SPECIFIC MEDIATORS IS DIFFERENTIALLY REGULATED VIA THE HISTAMINE H_4 RECEPTOR

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Monocyte-derived macrophages provide immediate defense against invading agents by their ability to phagocytose microorganisms and apoptotic cells e.g. neutrophils; further by secreting a broad array of mediators thereby promoting the development of the adaptive immune system. In response to different micro-environmental stimuli macrophages show high gene expression plasticity, altering their phenotype and functional responses. In chronic allergic skin diseases macrophages are attracted into tissues and are exposed mainly to Th2 cytokines and histamine, which are released in the skin of patients suffering from atopic dermatitis.

In this study, we investigated the role of the histamine receptors, in particular that of the histamine H_4 receptor (H_4R), during the differentiation of macrophages in presence of macrophage colony-stimulating factor (M-CSF) and on fully differentiated IL-4 activated so called M2 macrophages.

Measuring the H_4R mRNA expression at several time points during differentiation, we observed that H_1R and H_4R mRNA was expressed in monocytes and peaked on day 3 under differentiation with M-CSF. Activating the macrophages with IL-4 at several time points led to a further up-regulation of H_4R mRNA expression after 24 h. Furthermore, fully differentiated—or IL-4 activated macrophages—were stimulated with either histamine or the H_4R agonist ST1006. The mRNA expression or cell free supernatants were analyzed by quantitative real-time PCR and ELISA respectively. We observed a down-regulation of CCL2 production at early time points mainly in fully differentiated macrophages by stimulating the H_4R. Investigating the production of the chemokine CCL22, we detected an up-regulation after the differentiation of macrophages in presence of histamine. Since IL-4 up-regulated CCL22 production, we found higher levels of CCL22 mRNA expression and protein in IL-4 activated macrophages. Interestingly, pre-incubation with histamine led to a down-regulation of CCL22 production in IL-4 activated macrophages. A down-regulation of MMP9 production was also detected in IL-4 activated macrophages upon pre-stimulation with either histamine or the H_4R agonist ST1006.

To sum up, we could show that, dependent on the status of differentiation or activation, histamine and the H_4R agonist ST1006 led to a differential expression of M2 macrophage-specific mediators which might influence the course of allergic skin diseases.

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NO EVIDENCE FOR A ROLE OF THE HISTAMINE H_4-RECEPTOR IN CHRONIC DSS-INDUCED COLITIS

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In intestinal samples of inflammatory bowel diseases (IBD) from patients as well as from animal models, histamine is found in relatively high concentrations. dextran sulfate sodium (DSS)-induced colitis is a mouse model of IBD. Antagonists of the histamine H_4-receptor (H_4R) as well as genetic deletion of the H_4R significantly reduce symptoms of acute DSS-induced colitis in mice. In the present study we aimed to analyze a possible role of the H_4R in the model of chronic DSS-induced colitis in mice. Chronic colitis was induced in BALB/cJ mice, either wild-type or genetically H_4R-deficient ($H_4R^{-/-}$), by 4 cycles of feeding water supplemented with 2 % [w/v] DSS for 7 days. The DSS-cycles were separated by periods of 10 days with pure water alimentation. Control mice always received water without supplementation. Body weights of the mice were recorded every day. One day after the last DSS-cycle mice

were sacrificed and sera, caeca, colons, and mesenterial lymph nodes were prepared. Caeca and colons were histologically analyzed. Sera and supernatants of in vitro αCD3-stimulated lymph node cells were analyzed for cytokine expression. DSS-feeding induced a dramatic weight loss in wild-type mice, which recovered in the water-only interim periods. In H_4R-deficient mice weight loss and gain was very similar to that in wild-type mice. In the colon walls, inflammation as detected by histology was lower as compared to that observed in the acute colitis model. Moreover, no differences between wild type and $H_4R^{-/-}$ mice were observed. Similarly, concentrations of IL-6, IL-10, and MIP-2 in sera and in supernatants of in vitro αCD3-stimulated lymph node cells were lower than that observed in the acute model, and without differences between control and DSS-fed wild-type and H_4R-deficient mice. We conclude that the H_4R, which is involved in the regulation of acute DSS-induced colonic inflammation, has no impact on the pathogenesis of the chronic DSS-induced colitis model in Balb/c mice.

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EFFECTS OF SYSTEMIC TREAMENT WITH H_1 AND H_4 RECEPTOR ANTAGONISTS IN A MURINE OVALBUMIN-INDUCED ATOPIC DERMATITIS MODEL

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H_4R antagonists are considered as potential therapeutic agents for the treatment of allergic disorders such as atopic dermatitis (AD). The aim of this study was to assess the efficacy of H_4R and H_1R antagonists in a murine model of AD.

AD-like lesions were induced by repeated epicutaneous application of ovalbumin (OVA). Antagonists were given during the antigen challenge phase of the disease. H_4R antagonists JNJ 39758979 (20 and 50 mg/kg) and JNJ 28307474 (20 mg/kg) were administered orally two or three times daily. The H_1R antagonist mepyramine (30 mg/kg) was given i.p. twice daily. In a second set of experiments, JNJ 28747430 was also given during sensitisation, to analyse if the timing of drug application has an impact on the efficacy. Clinical skin score, serum level of OVA-specific IgE, epidermal thickness and infiltration of inflammatory cells, total cell count in spleen and axillary lymph nodes were analysed. Furthermore the cellular profile in the lymph nodes was analysed by FACS. Statistics were performed with GraphPad Prism 6 ($n = 6$, $p < 0.05$).

None of the treatments improved the dermatitis severity or the serum level of IgE. However, the combined treatment during sensitization and challenge modulated the total number of splenocytes and lymph node cells and decreased the inflammatory cell influx at lesional skin sites. Whereas the treatment with H_4R antagonists during the challenge only reduced the total number of splenocytes and increased the amount of macrophages in the draining lymph nodes; the H_1R antagonist decreased the number of B cells and CD4+ and CD8+ T cells in the lymph nodes. These results are in contrast to H_4R knockout mice which showed a remarkable amelioration of clinical signs in the OVA-model. Thus, it is important to clarify whether it is necessary to block the H_4R during sensitization and challenge, because pharmacological interventions in patients occur typically after the establishment of the disease. In addition, pharmacokinetic and pharmacodynamic properties might be responsible for the lack of effect.

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SEQUENTIAL APPLICATION OF LIGAND AND STRUCTURE-BASED MODELLING APPROACHES TO INDEX CHEMICALS FOR THEIR hH_4R ANTAGONISM

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Identification of bioactive chemicals in wet lab is very expensive and a time consuming process. In the present study, we describe how using in silico techniques could facilitate achieving such a goal. Aiming to increase the hit rate for selecting bioactive chemicals in the virtual screening process, we applied sequentially ligand-based modelling techniques followed by structure-based modelling techniques.

Two ligand-based chemoinformatics techniques, the Intelligent Learning Engine (ILE) and Iterative Stochastic Elimination approach (ISE), were applied to index chemicals for their human Histamine H_4 Receptor (hH_4R) antagonism. The hH_4R has attracted considerable interest as a potential target for the treatment of several inflammatory, allergic and autoimmune disorders, as well as for analgesic activity. To date no hH_4R ligands have reached the market and there is unmet need for developingnew selective ligands.

An application of the selected models on external test set composed of more than one hundred and sixty hH_4R antagonists picked from the chEMBL database gave an enrichment factor above sixteen. A virtual high throughput screening of the ZINC database was carried out, leading to approximately four thousand structures highly indexed as H_4R antagonists candidates. Next, a series of 3D models of the hH_4R were generated by molecular modelling and molecular dynamics simulations performed in fully atomistic lipid membranes. The efficacy of the hH_4R 3D models in discriminating between actives and non-actives were checked and the 3D model with the best performance was chosen for further docking studies performed with the focused library. Chemicals with exhibiting high scores may consequently provide useful starting points for drug design and validation via wet experiments.

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STRAIN-DEPENDENT UPREGULATION OF SPINAL CORD H_4 HISTAMINE RECEPTOR IN A RODENT MODEL OF NEUROPATHIC PAIN

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There is growing evidence for the role of the histamine H_4 receptor (H_4R) in sensory neurotransmission and its importance as a novel analgesic target for a range of acute and chronic pain states. We are currently working on establishing the relationship between affective state, neuropathic pain and the H_4R. This links to our previously reported studies using our novel open space anxiety tests and showing that Balb/C and CD1 display distinct patterns of affective behaviour. Here, neuropathic pain in mice (Balb/C and CD1, $n = 3$-5) and rats (Wistar, $n = 4$) was induced by speared nerve injury (SNI) or by chronic constriction injury (CCI), respectively. Conventional immunohistochemistry and quantitative immunoblotting were used to determine expression of H_4R at different levels of nervous system. Based on immunofluorescence approaches, we have collected evidence showing the presence of the H_4R on sensory fibres of sciatic nerve and in the skin, and in the neurons of spinal dorsal horn and DRG. Using immunoblotting, we provided preliminary evidence for the upregulation of the rat H_4 receptor protein level in the skin, but not the spinal cord dorsal horn, as measured 1 day after CCI in rats. In mice, however, our results indicated that nerve injury (SNI) led to a significant increase (48.8 ± 7.2 %; $F_{(2,7)} = 17.4$, $p = 0.002$) in the spinal dorsal horn protein level for H_4R in Balb/C, but not in CD1, as measured 7 days after the injury. While Balb/C mice have been shown previously to display a heightened level of innate anxiety compared to CD1, this approach now permits us to investigate the potential relationship between H_4R expression, anxiety level and chronic neuropathic pain. Based on our observation, we can hypothesize that this result is a direct response to the injury and/or heightened anxiety, given no significant increase was observed on the side contralateral to the SNI injury. This may potentially increase the pathways in which histamine and H_4R are implicated, broadening its neuronal effects. In summary, our results confirm a role for H_4R in the regulation and development of neuropathic pain, and show for the first time a potential involvement of H_4R in the correlation between neuropathic pain and the level of anxiety. Thus, our data may suggest H_4R as an important factor in the regulation of emotional response to pain.

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INVOLVEMENT OF THE HISTAMINE H_4 RECEPTOR IN THE REGULATION OF THYMIC STROMAL LYMPHOPOIETIN IN HUMAN KERATINOCYTES

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Thymic stromal lymphopoietin (TSLP) is a cytokine that is involved in the development and progression of allergic disease. It is mainly derived from epithelial cells that provide barrier functions, such as skin and gut in response to danger signals. Overexpression of TSLP in keratinocytes can provoke the development of a type 2 inflammatory response, leading to an atopic dermatitis-like phenotype. Additionally TSLP directly acts on sensory neurons and thereby triggers itch. Since histamine is also increased in lesions of inflammatory skin diseases, the aim of this study was to investigate possible effects of histamine as well as histamine receptor agonists and antagonists on TSLP production by human keratinocytes. We therefore stimulated hair and foreskin keratinocytes with the TLR3 ligand poly I:C and measured TSLP production at protein as well as mRNA level. We found that pre-incubation with histamine prior to challenge with poly I:C resulted in an increase of TSLP production compared to treatment with poly I:C alone. Experiments with different histamine receptor agonists [H_1: 2-pyridylethylamine; H_2: amthamine; H_4: ST-1006; H_2/H_4: 4-methylhistamine (4-MH)] revealed a dominant role for the H_4 receptor, as only pre-incubation with 4-MH significantly increased TSLP secretion in response to poly I:C. This effect was even greater than pre-

incubation with histamine. To verify that the effect is H_4 receptor-mediated, we blocked the H_4 receptor with the selective H_4 receptor antagonist JNJ7777120 prior to 4-MH and poly I:C treatment, and showed that the ability of 4-MH to potentiate TSLP production was inhibited.

Taken together, our data indicate a possible role for the H_4 receptor in the regulation of TSLP production by human keratinocytes. In allergic diseases, blocking the H_4 receptor could lead to a decrease in TSLP production and subsequently result in anti-inflammatory and anti-pruritic effects.

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IS PARP MODULATION INVOLVED IN HISTAMINE H_4R SIGNALING IN AN INFLAMMATORY MODEL OF LUNG FIBROSIS?

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Poly(ADP-ribose) polymerase (PARP) is a family of enzymes, involved in DNA repair and apoptosis. PARP-1 modulates the expression of inflammatory genes and is activated by reactive oxygen species.

We previously demonstrated that PARP inhibition attenuated oxidative stress damage, reducing the production of 8-OHdG in a model of bleomycin-induced lung fibrosis. Moreover, in the same animal model, the administration of a H_4R antagonist reduced oxidative stress in lung tissue, decreasing lung fibrosis. PARP-1 deficient mice exhibited reduced pulmonary fibrosis in response to bleomycin-induced lung injury.

The aim of the study is to evaluate the involvement of PARP-1 in H_4R signaling pathway through the administration of a H_4R antagonist and agonist in a model of bleomycin-induced lung fibrosis in PARP-1 *knock-out* (KO) and in *wild-type* (WT) mice.

C57BL/6 PARP-1 KO and WT mice were treated with bleomycin (0.05 IU) or saline intratracheally; VUF8430 (H_4R agonist, 2 mg/kg b.wt.) and JNJ7777120 (H_4R antagonist, 2.5 mg/kg b.wt.) were administered i.p for 21 days. Lung tissue was processed for PARylated protein content evaluation, oxidative stress (8-OHdG), as well as for histology of small bronchi. Moreover, iNOS and COX-2 isoforms were determined in homogenized lung tissue.

Our results showed that the administration of the H_4R antagonist in PARP-1 KO mice reduces PARylated protein content (1.73 ± 0.15 OD bleo + JNJ vs 2.25 ± 0.12 OD bleo + vehicle), decreases the amount of 8-OHdG (10.5 ± 1.5 vs 15.04 ± 1.3 ng/μg of DNA), an important marker of oxidative stress, and exerts an anti-inflammatory effect, reducing the expression of iNOS and COX-2 ($p < 0.05$). The treatment reduces the thickness of smooth muscle layer (15.67 ± 2.33 vs 22.33 ± 2.03 μm) and the goblet cell relative number (6.3 ± 0.88 vs 12.0 ± 3.1), both markers of bronchial remodeling, and the collagen deposition, a functional parameter of fibrosis. The administration of the H_4R agonist was less effective in the reduction of inflammation.

These results suggest that PARylation is important for the pathogenesis of pulmonary fibrosis and suggest that PARP-1 and H_4R are both involved in the signaling pathways activated during inflammatory and fibrotic processes. The combination of PARP-1 deficiency and H_4R antagonist treatment exerts an anti-inflammatory and anti-fibrotic effect, reducing bronchoconstriction and airway remodeling by decreasing inflammation and oxidative stress.

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ACTIVITY PROFILE AND DRUG-LIKENESS PROPERTIES OF THE MOST POTENT (4-METHYLPIPERAZIN-1-YL)-1,3,5-TRIAZIN-2-AMINE STYRYL DERIVATIVES

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Histamine mediates most of its function through binding to four well-described histamine receptor subtypes, designated as H_1–H_4. The youngest member of the family—the histamine H_4 receptor (H_4R), was discovered and cloned in 2000/2001 by several independent research groups, shows the highest sequence homology to the H_3R. It is assumed, that the H_4R could be involved in inflammatory processes and immune responses, because of its preferential expression in various cells of the immune system. The potential therapeutic effect of H_4R antagonists/inverse agonists in animal models of acute inflammations, allergic rhinitis, asthma or pruritus has been demonstrated. However, the physiological role of the H_4R is not yet clear, and thus new, potent and selective ligands are required to investigate its action.

The aim of this study was to evaluate the efficacy at H_4R and selectivity over the homologous histamine H_3 receptor for the most potent H_4R ligands: JN13/4-[(*E*)-2-(3-methylphenyl)ethenyl]-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine/and JN25/(4-[(*E*)-2-(3-chlorophenyl)-ethenyl]-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine/ selected from the newly obtained series of (4-methyl-

piperazin-1-yl)-1,3,5-triazin-2-amine derivatives with different styryl substituents in triazine 6-position.

The intrinsic activity of these structures was determined in a cAMP accumulation assay in HEK cells expressing recombinant human H_4R. Compounds were additionally tested for their selectivity over H_3R in a radioligand binding assay using [^{3}H]N^{α}-methylhistamine as radioligand. Furthermore, metabolic stability (in silico and using HLMs), the effect on CYP3A4 activity and the antiproliferative effect (against HEK293 and IMR32 cell lines) for JN25 were estimated.

The results showed that these derivatives may be considered as promising structures for further studies. Both JN13 and JN25 are selective antagonists at histamine H_4 receptors. Moreover, the potential structures of two JN25 metabolites were determined. JN25 showed no cytotoxicity against both HEK293 and IMR32 cell lines and was found to be a weak CYP3A4 inhibitor.

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Histamine H_3 receptor

HISTAMINE H_3 RECEPTOR BLOCKADE RESULTS IN INTRAOCULAR PRESSURE AND OXIDATIVE STRESS REDUCTION IN A RABBIT MODEL OF GLAUCOMA

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Elevated intraocular pressure (IOP) is the major risk factor for the development of glaucoma, therefore the reduction of IOP is considered the mainstream of glaucoma therapy. The present work is focused on evaluation of the effects of selective histamine H_3 receptor drugs in reducing IOP, oxidative stress and improving ocular vascular perfusion in different models of glaucoma.

Elevated IOP was obtained by the injection of 50 µl of hypertonic saline into the vitreous or carbomer 100 µl (0.1 %) in the anterior chamber of New Zealand albino rabbits'eyes. The IOP measurements were performed prior to saline or carbomer injection (baseline), immediately before drug dosing (pre-treatment) and 1–4 h after in the acute saline model and every day for 2 weeks in chronic carbomer model. Biochemical and morphological changes were also assessed in the aqueous humour samples, in retinal and ciliary body biopsies. Immunohistochemistry and Western blot analyses were performed to localize the H_3 receptors.

IOP rose from 13.4 ± 2.7 mmHg at baseline to 36.6 ± 8 mmHg after hypertonic saline injection and from 12.2 ± 2.1 to 34.4 ± 4.2 after carbomer injection and remained stable for 2 weeks. Histamine H_3 receptors, evaluated with immunohistochemistry and Western blot, were mainly expressed in ciliary bodies. DL-76 (0.5–1 % solutions), a novel histamine H_3 (HH3) inverse agonist and ciproxifan (1 % solution), a well characterized HH3 antagonist, lowered IOP at 60 and 120 min after saline injection and in carbomer ocular hypertension (p value <0.01 at 120 min; $n = 6$ in saline model; $n = 6$ in carbomer model). After repeated administrations of DL-76 and ciproxifan, both administered at 1 % solution in carbomer model, mean resistance index (RI) of ophthalmic arteries and oxidative stress markers were significantly decreased as well as retinal ganglion cell loss (p value <0.05). HH3 receptors represent an interesting new therapeutic target for the development of new drugs for glaucoma treatment.

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ALOYSIA TRIPHYLLA ESSENTIAL OIL AS A NEW NATURAL SOURCE OF HISTAMINE H_3 RECEPTOR ISOFORM LIGAND CANDIDATES?

R. Abuhamdah, S. Abuhamdah, N. Lethbridg, P.L. Chazot

Selective antagonism of centrally localized histamine H_3 receptors has been shown to enhance the release of a wide spectrum of important neurotransmitters including acetylcholine, gamma-aminobutyric acid, dopamine and noradrenaline, among others. These play fundamental roles in neurological processes, in an output-dependent manner. We have growing evidence that H_3R 445 (full length version), 365 and 329 isoforms exist in human brain based on the use of our panel of unique human isoform-specific antibodies (e.g. Shan et al. 2012). The physiological and clinical relevance of H_3R isoforms has yet to be understood. Using these immunological probes, we also have preliminary evidence that hH_3R 329 and 445 are elevated, while 365 remains largely unchanged, in the putamen from both Parkinson's disease and Lewy Body dementia cases vs age-matched normal controls. *Aloysia triphylla* is a perennial, bushy plant, originally from South America, and grown in various areas in the Middle East, including Jordan. It is cultivated mainly due to the lemon-like aroma emitted from its leaves that are utilized for the preparation of herbal tea, which is reputed to have antispasmodic, antipyretic, sedative and digestive properties. It has a long history of folk uses in treating asthma, spasms, cold, fever, flatulence, colic, diarrhoea, indigestion, insomnia and anxiety. We have defined some of the neuropharmacological properties of this plant essential oil and extracts, and shown them to possess a range of interesting properties (e.g., anti-cholinesterase, neuronal nicotinic binding, metal-chelation and anti-oxidant properties) that make it well-suited for as a candidate for neurological treatment. Herein, we report new evidence that freshly prepared Jordanian-sourced *Aloysia* essential oil (identity validated by Kew Gardens Jodrell Laboratory, UK) contains component(s) that appear to bind human H_3 receptors, in a differential isoform-dependent manner (apparent pIC_{50} values, mg/ml, H_3R 329 (3.01 ± 0.07), H_3R 365 (1.67 ± 0.07), H_3R 445 (1.12 ± 0.04) based on [^{3}H] GSK189294 competition binding assays; mean values $\pm$ SD for $n = 3$ separate experiments). We are currently analysing in vivo effects of the *Aloysia* essential oil, and modelling components within the *Aloysia* essential oil to known H_3R ligand chemotypes.

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EFFECT OF HISTAMINE H_3 RECEPTOR ACTIVATION ON THE INTRACELLULAR CONCENTRATION OF CALCIUM IONS IN RAT STRIATAL AND PALLIDAL SYNAPTOSOMES AND STRIATAL NEURONS IN PRIMARY CULTURE

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The histamine H_3 receptor (H_3R) is abundantly expressed in the central nervous system where it regulates at the pre- and post-synaptic levels several functions. Through their coupling to Gαi/o proteins H_3R activation triggers several intracellular effects, most notably the regulation of cAMP formation and the inhibition of N- and P/Q-type voltage-activated Ca^{2+} channels. In heterologous expression systems H_3R activation also stimulates phospholipase C (PLC) resulting in an increase in the intracellular concentration of calcium ions ($[Ca^{2+}]i$). In this work, we aim to determine whether H_3R activation induces Ca^{2+} mobilization from intracellular stores in native systems, namely isolated nerve terminals (synaptosomes) and neurons in primary culture. We confirmed that the activation of the human H_3R (445 amino acids) resulted in PLC activation and thus in both inositol 1,4,5-trisphosphate (IP_3) formation and calcium mobilization in CHO-K1 cells stably transfected. Striatal GABAergic neurons that project to the globus pallidus or the substantia nigra pars reticulata express high levels of H_3Rs. In synaptosomes from rat striatum or globus pallidus H_3R activation induced IP_3 formation but failed to increase the $[Ca^{2+}]i$, presumably by the lack of endoplasmic reticulum in the nerve terminals. In striatal neurons in primary culture H_3R activation resulted in IP_3 formation, as well as an increase in the $[Ca^{2+}]i$ in 6 out of 30 cells in which depolarization with KCl (100 mM) also raised the $[Ca^{2+}]_i$. These results indicate that in a subpopulation of striatal neurons H_3R activation stimulates the $PLC/IP_3/Ca^{2+}$ pathway. The lack of response in a large fraction of the analyzed cells could be explained by the low sensitivity of the PLCβ1 isoform, expressed by striatal projection neurons, to Gβγ complexes produced in response to H_3R activation.

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THE HISTAMINE H_3 RECEPTOR ANTAGONIST DL77 REDUCES VOLUNTARY ALCOHOL INTAKE AND ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE IN MICE

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Histamine H_3 receptors (H_3R) have been implicated in modulating ethanol intake and preference in laboratory animals. The novel non-imidazole H_3R antagonist DL77 shows high in vivo potency as well as in vitro antagonist affinity with ED_{50} of 2.1 ± 0.2 mg/kg and 8.4 ± 1.3 [nM], respectively. In the present study, and applying an unlimited access two-bottle choice procedure, the anti-alcohol effects of the H_3R antagonist, DL77 (0, 3, 10 and 30 mg/k; i.p.), were investigated in adult C57BL/6 mice. Effects for this compound on voluntary alcohol intake and preference, as well as on total fluid intake were evaluated. Results have shown that DL77, dose-dependently, reduced both ethanol intake and preference. These effects were very selective as both saccharin and quinine, used to control for taste sensitivity, and intake were not affected following DL77 pre-application. Moreover, systemic administration of DL77 (10 mg/kg) during acquisition inhibited ethanol-induced conditioned-place preference (EtOH-CPP) as measured using an unbiased protocol. The anti-alcohol activity observed for DL77 were abrogated when mice were pre-treated with the selective H_3R agonist *R*-(α)-methylhistamine (RAMH) (10 mg/kg), or with the CNS penetrant H_1R antagonist Pyrilamine (PYR) (10 mg/kg). These results suggest that DL77 has a role in two in vivo effects of ethanol. Therefore, signalling via H_3R is essential for ethanol-related consumption and conditioned reward and may represent a novel therapeutic pharmacological target to tackle ethanol abuse and alcoholism.

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EVIDENCE FOR THE INVOLVEMENT OF HISTAMINE AND H_3R IN THE CONTROL OF MOTOR FUNCTIONS IN HEMIPARKINSONIAN MICE

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Brain histamine is recognized as one of the modulatory neurotransmitter systems controlling basal ganglia function. Alterations in histaminergic system have been found post mortem in Parkinson's disease (PD) patients and in experimental PD animal models. Herein, we examined the role histamine and its H_3 receptor in hemiparkinsonian PD mouse model induced by 6-hydroxydopamine injection (6 μg) into the dorsal striatum. We found that histidine

decarboxylase knockout mice (HDC KO) show increased ipsilater amphetamine-induced rotational behavior compared to their control wildtypes (WT) animals ($p = 0.0064$ 2-way ANOVA, $n = 8$-12) suggesting a more severe lesion of dopaminergic cells in HDC KO mice. However, tissue contents of striatal dopamine were similar in wildtype and HDC KO mice. Interestingly, also the histamine content was reduced at the site of lesion in wildtype mice suggesting cell death of histamine neurons by 6-OHDA. We also tested the effect of novel H_3R antagonist JNJ-39200675 (3 and 10 mg/kg) on amphetamine-induced rotational behavior and found that both doses of the H_3R antagonist inhibited rotations ($p = 0.0019$, 2-way ANOVA; $n = 7$–8). This indicates that H_3R antagonist is able to improve the motor imbalance in hemiparkinsonian model. However, 10 mg/kg of JNJ-39200675 inhibited the use of contralateral paw (weak paw) in a cylinder test. In conclusion, these findings suggest a role for brain histamine and H_3R in the control of motor functions in animal PD model.

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H_1 AND H_3 HISTAMINE RECEPTORS AND CHEMOKINE SYNTHESIS

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It is well known that histamine via activation of H_1–H_4 receptors induces multiple immunological responses. Most of the effects depend on the release of different cytokines and chemokines. Recent investigations have shown the key role not only to be through cytokines and CC chemokines, but also through monocyte and other cell types, attraction of T cells, eosinophils and basophils. Chemokines are involved in the regulation of cell migration into the allergic inflammatory tissues. The goal of this investigation was to study the involvement of histamine H_1 and H_3 receptors in the production of the main chemokines (Eotaxin, RANTES, MCP-1, MIP-1α, MIP-1β) by PBMC from healthy male donors. PBMC of 5 healthy donors were cultured in the presence of the H_1 agonist (2-methylhistamine, 10^{-5}M) or the H_3 antagonist (Ciproxifan, 10^{-5}M). The concentration of chemokines (Eotaxin, RANTES, MCP-1, MIP-1α, MIP-1β) in 48-hour supernatants was investigated by Multiplex assays using Luminex xMAP technology. Activation of H_1 receptors by 2-methylhistamine and blocking of H_3 receptors by Ciproxifan resulted in similar effects. Both agents greatly increased the synthesis of the chemokines Eotaxin, RANTES, MIP-1α, MIP-1b by between 2–8-fold. For example: Eotaxin increased from 6.8 ± 0.73 up to 11.8 ± 0.75 pg/ml after culturing with 2-methylhistamine and up to 13.2 ± 1.92 ($p < 0.05$) after culturing with Ciproxifan; RANTES increased after culturing with H_1 agonist and $H_{3/4}$ antagonist from 140.8 ± 45.6 up to 356.4 ± 130.6 and 710.3 ± 226.5 pg/ml $p < 0.05$, respectively. At the same time there was little or no effect of 2-methylhistamine or Ciproxifan on the production of MCP-1 by PBMC ($p > 0.05$). The study showed that H_1 as well as H_3 histamine receptors are involved in chemokine synthesis by mononuclear cells and this effect maybe important for the further investigations of the mechanisms of regulation of inflammatory disorders via histamine receptors.

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THE PYRIDINIUM-BASED FLUORESCENT HISTAMINE H_3 RECEPTOR LIGAND ST1516 AS USEFUL PHARMACOLOGICAL TOOL

B. Sadek, M. Tomasch, P. Freund, J.S. Schwed, M. Heilemann, H. Stark

A novel fluorescent pyridinium-based ligand (ST1516) at human histamine H_3 receptors (hH3R) has been designed, synthesized, and characterized. The fluorescent compound is described as a non-imidazole cationic H_3R antagonist/inverse agonist incorporating a 4-((*1E,3E*)-4-(4-(dimethylamino)phenyl)buta-1,3-dienyl)-2,6-dimethylpyridinium motif. ST1516 has been synthesized starting from 3-(piperidin-1-yl) propane-1-amine in a coupling reaction with the appropriate pyrylium derivative.

The compound displayed moderate hH_3R affinity and high Stokes shift. Fluorescence labeling of hH_3R with ST1516 on human glioblastom (U251) and on osteo carcinom (U2OS) cells shows emission maxima about 688 nm as a blue fluorescent derivative. The synthesized pyridinium-based ligand could be used to visualize hH_3R proteins in stably transfected cells as well as in cell lines using confocal laser scanning fluorescence microscopy taking advantage of the new stochastic optical reconstruction microscopy (STORM) technique.

In contrast to the recently described highly useful fluorescent hH_3R ligand Bodilisant, the novel fluorescent compound ST1516 possesses different physicochemical

properties with a potential to be used as alternative pharmacological tool for hH_3R visualization in different tissues.

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ANALOGS AND DERIVATIVES OF PIPERIDINYL-PENTOXYPHENYL COMPOUNDS AS HISTAMINE H_3 RECEPTOR LIGANDS

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Histamine H_3 receptors (H_3Rs) are one of the four known histamine receptors. They are mostly expressed in the CNS and regulate the release of histamine itself and other neurotransmitters (e.g., acetylcholine, noradrenaline, dopamine and serotonin). Consequently H_3R antagonists/inverse agonists constitute attractive targets in the search for new drugs. Preclinical data indicate their potential utility in the treatment of various central nervous system (CNS), metabolic, pain and allergic disorders. So far, many structurally diverse H_3R ligands have been synthesized and pharmacologically evaluated.

As a continuation of our previous work, we synthesized a series of (methyl) (homo) piperidinyl-pentoxyphenyl analogs of our lead structure DL76 (1-[3-(4-*tert*-butylphenoxy)propyl]piperidine). Compounds were screened for their binding affinities at recombinant human H_3Rs stably expressed in HEK-239 cells and exhibited good affinities (K_i $hH_3R < 100$ nM).

Several compounds and DL76 were chosen for further pharmacological studies checking their extended profile of activity (e.g. in vitro in cAMP assay, in vivo in dipsogenia). Selected compounds were also tested in the Passive Avoidance Test and showed the ability to reverse the scopolamine-induced impairment on memory and learning behaviour.

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PHARMACOLOGICAL CHARACTERIZATION OF ZEBRAFISH H_3R-LIKE RECEPTORS

D.A. McNaught-Flores, H. Vischer, H. Puttonen, Y-C. Chen, P. Panula, R. Leurs

The zebrafish (*Danio rerio*) has become a widely used in vivo model for the study of vertebrate development and gene function. This organism has become a powerful tool for e.g. genetic studies, drug screening, and CNS development and neurological diseases.

The zebrafish histaminergic system resembles that of other vertebrates with a similar localization of mammalian histamine containing neurons. Peitsaro et al., in 2007 cloned three histamine receptors from zebrafish brain that have a 40–50 % homology with the mammalian counterparts. However, these receptors have been briefly characterized and recently two more H_3-like receptors also have been cloned. In this poster we will present first data on the zebrafish H_3-like receptors. We reported on the sequence analysis, receptor expression and compare the pharmacology with the human H_3 receptor on the basis of results from radioligand binding studies and signal transduction studies in recombinant cell systems.

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ACTIVATION OF ENDOGENOUS PURINERGIC RECEPTORS INDUCES PKC-MEDIATED DESENSITIZATION OF HUMAN HISTAMINE H_3 RECEPTORS EXPRESSED IN CHO-K1 CELLS

W. Montejo-López, N. Rivera-Ramírez, J. Escamilla-Sánchez, U. García-Hernández, J.-A. Arias-Montaño

Desensitization is a major mechanism to regulate the functional response of G protein-coupled receptors (GPCRs), by modifying receptor signaling efficacy and expression at cell surfaces. Homologous desensitization is triggered by the phosphorylation of activated receptors by GPCR kinases (GRKs), whereas the heterologous process leads to the loss of responsiveness of receptors not activated by agonist, and frequently involves GPCR phosphorylation by second messenger-activated kinases such as PKA and PKC. Our laboratory recently showed that the human histamine H_3 receptor of 445 amino acids (hH_3R_{445}), stably expressed in CHO-K1 cells, experienced homologous desensitization. In this work we aimed to determine whether the activation of endogenous purinergic

receptors, coupled to $G\alpha_{q/11}$ proteins and thus to phospholipase C stimulation and PKC activation, induced heterologous desensitization of the hH_3R_{445} in CHO-K1-hH_3R_{445} cells. Bioinformatic analysis indicated the presence of residues susceptible of PKC-mediated phosphorylation on the third intracellular loop (11 Ser/2 Thr) and the carboxyl terminus (1 Ser) of the hH_3R_{445}. Direct PKC activation by the phorbol ester TPA (200 nM) abolished the H_3R-mediated inhibition of forskolin-induced cAMP accumulation ($n = 4$ experiments), and this effect was blocked by 10 μM Ro-31-8220 (an inhibitor of PKC isoforms α, βI, βII, γ and ε) or by 10 μM Gö-6976 (an inhibitor of the α and β isoenzymes) ($n = 5$ experiments). The activation of purinergic receptors by ATP (10 μM) resulted in a marked increase in the intracellular Ca^{2+} concentration due to release from intracellular stores (Individual cells representative of 15 determinations with different cell batches). Pre-incubation with 10 μM ATP prevented the inhibitory effect of H_3R activation on forskolin-stimulated cAMP accumulation, and this effect was also blocked by either Ro-31-8220 or Gö-6976 ($n = 3$ experiments. $^*P < 0.001$ when compared with forskolin alone, ANOVA and Tukey's test). These results indicate that the hH_3R_{445} undergoes heterologous desensitization following the activation of receptors coupled to the phospholipase C/Ca^{2+}/diacylglycerol/PKC pathway.

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Molecular, genetic and chemical aspects of histamine, its receptors and enzymes

G PROTEIN-COUPLED RECEPTOR KINASES: FROM GPCR MODULATION TO SIGNALLING HUBS

F. Mayor Jr., C. Murga, P. Penela, C.Ribas

G protein-coupled receptor kinases (GRKs) were initially identified as negative regulators of G protein-coupled receptors (GPCR). However, recent evidence is unveiling novel roles for these proteins, in particular for the ubiquitous and essential GRK2 isoform, which is emerging as a key signalling hub. Importantly, GRK2 levels are altered in human pathologies such as inflammation, cancer, and cardiovascular/metabolic diseases, suggesting that it might play a relevant pathophysiological role.

Activated GPCR become specifically phosphorylated by GRKs, what promotes the association of β-arrestins, leading to uncoupling from G proteins and GPCR internalization. In turn, arrestins act as scaffold proteins triggering the modulation of additional signalling cascades by GPCR. Therefore, changes in GRK2 levels or activity may control the balance/bias between G protein-dependent vs GRK/β-arrestin-dependent cascades. In addition, GRK2 can also impact cell signaling networks by directly phosphorylating non-GPCR substrates (Smads, HDAC6, ezrin, IRS1, or p38 Mapk, among others) and/or dynamically interacting with other partners (for instance Gαq, PI3 K/Akt, GIT1, MEK, IRS1, EPAC, Pin1 or Mdm2) thus acting as an effector of transduction cascades. Using both cellular and animal models with altered GRK2 dosage or functionality, we have shown that combinations of such canonical and non-canonical functions underlie the participation of GRK2 in the modulation of cell migration, angiogenesis, tumour progression and hypertension or insulin resistance in specific cell types or physiological contexts.

It is also worth noting that GRK2 is unique among GRKs in its ability to interact with Gαq. This would convey an additional level of regulation of Gq-GPCR signal cascades (increasingly implicated in metabolic/cardiovascular diseases and in tumour promotion) by blocking interaction of Gαq with known effectors as phospholipase Cβ (PLCβ) or p63RhoGEF. Our group has described a novel Gq-GPCR signalling axis that relies upon the interaction between Gαq and two novel effectors (PKCζ and MEK5), which is key for ERK5 MAPK activation. Such novel interactions of Gαq with PB1-domain containing proteins such as PKCζ, and its modulation by GRK2, open new avenues for the control of cellular processes by GPCR.

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TOWARDS THIRD GENERATION ANTIHISTAMINES: FACT OR FICTION?

R. Leurs

The histamine receptor family has so far been very successful as target for blockbuster drugs. With the first and second generation "antihistamines" (H_1R antagonists/inverse agonists) and the Nobel-prize awarded H_2R antagonists/inverse agonists, the histamine H_1R and H_2R receptors are regarded as classical targets from the blockbuster era. The third histamine receptor has for long been studied as drug target in CNS disorders, whereas the elucidation of the human genome and the subsequent expansion of the histamine receptor family to 4 members offered additional opportunities to discover new (patho)-physiological roles of histamine.

In this talk, I discussed the options to come to new (third, fourth?) generation of "antihistamines" in light of all the recent insights in GPCR-ligand interactions, like e.g. target residence time, biased signaling, and polypharmacology.

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LIGAND BINDING KINETICS FOR THE H_1 RECEPTOR—PROPERTIES OR LONG BINDERS

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Ligand-receptor binding kinetics may be considered better predictors then affinity for in vivo effects and could therefore improve drug design. Binding kinetics for several H_1 antihistamines were analyzed via radioligand binding studies. Data suggested that second generation antihistamines have longer complex lifetimes with the receptor than first generation antihistamines suggesting a possible relationship between ligand-receptor binding kinetics and success in the clinic. Mutational analysis and analysis of structurally related compounds helped to pinpoint binding sites and the structural differences between H_1 ligands which determine the kinetic profile of ligand binding to the H_1 receptor. Results show how the different factors, including radioligand binding and tailored molecular dynamic experiments, are a powerful way to mechanistically explain how GPCR ligands differ in their binding kinetics for their receptor, providing a way for optimized ligand design.

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HISTAMINE AFFECTS THE GLOMERULAR SLIT DIAPHRAGM INTEGRITY THROUGH HISTAMINE H_1RECEPTORS

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The glomerular slit diaphragm (GSD) represents the junctional structure linking the interdigitating foot processes of podocytes. Its function in regulating the ultrafiltration coefficient (kf) is guaranteed by both tight (mostly zonula occludens-1, ZO-1) and P-cadherin-based adherens junctions. Although an effect of histamine on kf has been already reported, the underlying mechanism(s) have not yet been clarified completely.

Thus, this study aimed to evaluate whether histamine affects ZO-1 and P-cadherin expression in human immortalized podocytes.

mRNA and protein expression for all histamine receptors were evaluated by RT-PCR and immunofluorescence; IP3 and cAMP production evoked by histamine (3 pM-10 nM) were measured by TR-FRET. The effect of histamine on ZO-1, P-cadherin and vimentin expression was assessed through Real Time RT-PCR and immunoblotting. The integrity of the tight junctions was evaluated by electron microscopy.

Our data revealed the presence of H_1R, H_2R and H_4R, although only H_1R was predominantly localized to the membrane. Consistent with the morphological observations, histamine failed to modulate cAMP production, but elicited a sigmoidal increase in IP3 over the concentration range 3 pM-10 nM. Histamine exposure evoked a concentration-dependent reduction in both ZO-1 and P-cadherin and a parallel induction of vimentin mRNA expression with a maximum after 6 h (-50 ± 3, -54 ± 3 %, $+40 \pm 6$ %) and protein expression with a maximum after 8 h (-60 ± 3, -80 ± 2, $+100 \pm 10$ %). All of these effects were prevented by the selective H_1R antagonist chlorpheniramine maleate, but not by the H_2R antagonist ranitidine or the H_4R antagonist JNJ7777120 (10 μM). These observations were validated when junctional integrity was observed by electron microscopy.

In conclusion, our data demonstrate that histamine exerts a detrimental direct effect on GSD integrity through H_1R by decreasing both ZO-1 and P-cadherin expression, thus probably promoting an epithelial-mesenchymal transition, as suggested by the increase in vimentin expression.

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IN-SILICO MODELLING OF PRIMARY HYPEROXALURIA TYPE 1, A HUMAN INBORN ERROR OF LIVER METABOLISM, UNRAVELS A KEY ENZYME FOR HISTAMINE HOMEOSTASIS

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We applied a computational approach to simulate in silico Primary Hyperoxaluria Type I (PH1), an autosomal recessive inborn error of liver metabolism caused by deficiency of alanine:glyoxylate aminotransferase (AGT). The in silico model correctly predicted oxalate accumulation as well as alterations in other known PH1-related metabolites. Surprisingly, histamine was also predicted to be perturbed. We confirmed in vitro (Huh-7 cells, $p < 0.05$), in vivo ($Agxt^{-/-}$ mice compared to wild-type mice, at least $n = 5$ per group, $p < 0.05$) and in PH1 patients (PH1 patients harboring *AGXT* mutations, $n = 3$, compared to normal subjects, $n = 5$, $p < 0.05$) a strong reduction in histamine levels. Moreover, in AGT deficient mice reduced histamine resulted in decreased vascular permeability upon drug-induced histamine release ($n = 8$ per group, $p < 0.05$). In-depth analysis of the in silico model revealed that histamine reduction is caused by increased amino acid catabolism mediated by the glutamic-pyruvate transaminase (GPT also known as ALT). We predicted in silico and confirmed in vivo that overexpression of GPT normalises histamine levels ($n = 5$ per group, $p < 0.05$), restores vascular permeability ($n = 5$ per group, $p < 0.05$), and decreases urinary oxalate levels ($n = 5$ per group, $p < 0.05$). Our work demonstrates that genome-scale metabolic models are clinically relevant and able to link genotype to phenotype in metabolic disorders.

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MAMMALIAN HISTIDINE DECARBOXYLASE: FROM THE THIRTHIES TO THE XXI CENTURY

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Histamine is the decarboxylation product of L-histidine decarboxylase. In mammals, this is a PLP-dependent homodimeric enzyme (HDC, EC 4.1.1.27) that is only expressed in a reduced set of cell types (mainly mast cells, enterochromaffin-like cells and histaminergic neurons). The instability of the enzyme and the dispersed location of its producing cells in mammals make it very difficult to fully characterize, especially in the case of the human enzyme (hHDC). The first reports on the mammalian enzyme were published during the mid-thirties. Eighty years later, we have considerable knowledge about its quaternary structure, kinetic constants and the proteolytic systems acting on it, at least in vitro. This was the result of several international groups working on different aspects of the enzyme.

However, several questions still remain to be fully answered. From a structural perspective, there remains questions about the orientation of the substrate L-histidine in the catalytic center.This lack of information is delaying the development of new specific inhibitors. In addition, many questions remain to be answered in order to fully understand the complex cell type-specific regulation of hHDC expression and turnover, and its importance in many pathologies.

In this communication, we reviewed these 80 years of mammalian HDC research and summarize the major challenges to be met to move the science from the bench to the bedside. We lso presented strategies to reach these goal.

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VISIBILITY OF AMINE-RELATED ELEMENTS IN HUMAN DISEASOMES

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Biogenic amines (BA) are amino acid derivatives mainly synthetized by PLP-dependent decarboxylases and degraded by amino oxidases. Their biochemical pathways are linked to hubs of primary metabolism (i.e.: Acetyl-CoA and S-adenosyl methionine). Their degradation produces ROS and aldehydes. Many crosstalk events have been described among BA pathways with relevance in different pathophysiological problems. Polyamines have major metabolic roles in protein synthesis, whereas other BAs play key roles in intercellular communication. Alterations in biogenic amine (and biogenic amine-related macromolecule) patterns are involved to many pathological conditions: neoplasias, inflammatory and neuroinflammatory diseases, neurological diseases, among others, including a long list of rare diseases.

By using bioinformatics tools we have analyzed how the pathophysiological data regarding biogenic amines is reflected in the most important repositories. Results show a gap between information available in literature and the standardized information present in disease databanks. The gap is especially dramatic in the case of histamine, since there is no pathology related with histamine receptors in OMIM and Orphanet. PhenUMA (http://www.phenuma.uma.es) is a web tool that integrates phenotypical and functional information. However, the lack of information associated with histamine-related genes makes difficult to connect phenotypical information to these genes. To solve this, we have designed a workflow that makes use of text mining tools, biomedical ontologies, semantic similarities and data integration to retrieve information associated with histamine-related genes from literature. Our preliminary results show functional and phenotypic relationships among histamine receptors and dopamine receptors genes. This information could be included in an Amine Research Project for the development of a new version of PhenUMA, including information provided by text mining tools.

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NATURAL LIKENESS OF CHEMICALS AND DRUG DEVELOPMENT

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The majority of the currently marketed drugs are natural based products or their derivatives and there is an indication that these compounds are inherently better tolerated in the human body and have a greater chance to survive the drug development process. Furthermore, our previous report revealed that natural product-based chemical libraries have drug-like properties more than synthetic compound libraries. In the present study, we aim to introduce a new highly efficient rules-based filter to assess the natural likeness of chemicals using 1D/2D physicochemical properties. For this purpose we have applied the Iterative Stochastic Elimination approach (ISE) to index chemicals for their natural likeness. ISE is a generic tool used for solving NP hard problems and for scanning systems that can not be searched and optimized by deterministic methods due the the huge number of potions for these systems. The tests of

the current study have been carried out on highly diverse set of more than fifteen hundred natural products and nine thousand synthetic chemicals. These compounds have been randomly divided into two equal sets, namely a training set and a test set.We reported an analysis of the physicochemical properties and disclosed some filters capable of differentiating natural products and synthetic chemicals. Analyzing the histograms of Lipinski four descriptors shows that the mean vlaues for moleuclar weight, number of hydrogen bond acceptors and donors are less for synthetic chemicals compared to natural products. As well, natural products are more soluble in water compared to synthetic chemicals. Potential applications of the proposed indexing approach are many-fold. It could be incorporated into virtual screening processes of large chemical databases, and prioritization of compounds for purchase and high-throughput screening.

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CATALOGUING THE FUNCTIONAL EFFECTS OF GENOMIC VARIATION IN AND UPSTREAM OF HISTAMINE RELATED GENES

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Histamine is involved in a variety of biological processes, including the immune system, where it is involved in inflammation and allergy through mast cell degranulation, leading to increased permeability in nearby blood vessels. It functions by binding to G protein-coupled histamine receptors. The downstream effects of this binding are dependent on the specific histamine receptor bound, making regulation of their expression crucial to the correct physiological response.

We have studied the effects of genetic variants within and upstream of a number of genes involved in histamine related processes, including histamine receptors, genes involved in its metabolism, secretion and transport, and mast cell degranulation (e.g. histidine decarboxylase, histamine N-methyl transferase and diamine oxidase). SNP data was obtained from dbSNP; we then studied the function of these variants by examining (i) their effects on gene expression through the use of eQTL data, (ii) changes to transcription factor (TF) binding site motifs, (iii) overlap with regions of open chromatin, (iv) areas of TF binding according to ChIP-seq data. Associations with disease were also examined using public GWAS and 1000 Genome Project data.

Several SNPs close to or within histamine related genes were found to affect gene expression regulation, through effects on TFBS and related to chromatin structure. We also found SNPs associated with various diseases and phenotypes, including immune system disorders/processes such as monocyte outgrowth and IgG glycosylation, asthma, and some CNS related processes such as cognitive performance.

We have combined various data sources and found potentially important functional variants supported by different lines of evidence. Further study is required including genetic and functional study in appropriate patient populations and models.

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A TOOL FOR IDENTIFYING PUTATIVE SNPs IN NON-CODING GENOMIC REGIONS, APPLIED TO HISTAMINE RECEPTORS

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Genome wide association studies (GWAS) have been successful in finding genetic variants associated with disease, such as single nucleotide polymorphisms (SNPs). Almost 90 % of these variants have been found in non-coding genomic regions, and are likely to be involved in the regulation of gene expression. For example, enhancer regions, found upstream of genes influence gene expression levels through transcription factor binding, therefore genetic variation in these regions can affect expression levels by changing binding affinity. Thus, a tool able to search for and identify such variants, and assign putative functions, could be interesting in order to explain the effects of SNPs in non-coding regions.

We have built a workflow that looks at SNPs in non-coding regions of genes, and the 5′ regions upstream of the transcription start site. It ascribes putative function for these SNPs by using data on eQTL, chromatin modifications, DNA methylation and transcription factor binding motifs. It also looks at SNPs in linkage with disease associated SNPs using the NHGRI GWAS catalogue and the 1000 genomes data.

The workflow was used to analyse histamine genes, focussing on genes involved in the immune response such as the histamine receptor family, and genes involved in mast cell degranulation such as Bruton Agammaglobulinemia Tyrosine Kinase. We found a number of SNPs that affect transcription factor binding and gene expression, as well as SNPs associated with several traits, such as IgG glycosylation.

This tool can be used for a variety of potential functions, including target prioritization, the analysis of GWAS data, and to find out more about a gene or pathway of interest. We have applied it to the analysis of histamine genes and found potential new insights into their regulation. Future work includes making the workflow publicly available and applying it to the analysis of more genes and datasets through collaboration.

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Histamine in the cardiovascular and gastro-intestinal systems

HISTAMINE AND RELAXIN: AN INTRIGUING CONNECTION

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Histamine is released during cardiac anaphylaxis and ischemic-reperfusion injury, producing severe arrhythmias, cardio-depressive effects and coronary spasm.

Relaxin (RLX), formerly known for its effects on reproduction and pregnancy, has been shown to be a pleiotropic hormone, targeting numerous non-reproductive organs. Relaxin, at 10 ng/ml, promotes dilation of blood vessels in guinea pig isolated heart; inhibits the release of histamine; depresses aggregation of platelets induced by thrombin from 82 to 40 % of maximum ($p \leq 0.001$) and their release from megakaryocytes and contributes to the regulation of fluid balance.

Experimental studies performed in vascular and blood cell in vitro and in animal models of vascular dysfunction as well as pioneer clinical observations, have provided evidence that RLX prevents and/or improves cardiovascular diseases, such as ischemia–reperfusion and heart failure. Concerning the mechanisms of action of relaxin and histamine, stimulation of nitric oxide (NO) generation, with consequent rise in intracellular cyclic GMP (from 4 to 28 pg/mg of protein) production has been demonstrated to occur in the target cells and organs.

Dimaprit, a histamine H_2 receptor agonist, decreases the amount of histamine released during the first 5 min of cardiac anaphylaxis from 4.2 to 1.4 μg/g of tissue and controls the positive inotropic and chronotropic responses of the isolated heart taken from actively sensitized guinea pigs, an effect mediated by NO-cGMP pathway.

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PROTECTIVE EFFECTS OF HISTAMINE AND CLOZAPINE AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY AND HEPATOTOXICITY

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Radiation and chemotherapy are widely used treatments for cancer. Despite its anti-neoplastic effects, both can cause disabling normal tissue injury. We have reported that histamine (HA) acts as an effective radioprotective agent on highly radiosensitive tissues. We also demonstrated that the combination of HA and ionizing radiation or chemotherapy (doxorubicin, DOX) increased the anti-tumoral effects of both treatments on human breast cancer cell lines.

In the present work, we aimed to evaluate whether HA or clozapine (CLZ) are able to prevent the DOX-induced hepato and cardio-toxicity in two rodent species. Animals were divided into 6 groups: HA (5 mg/kg) and CLZ (1 mg/kg) groups received a daily *sc* injection starting 24 h before treatment with DOX (2 mg/kg, i.p.) 3 times a week for 2 weeks. Heart of DOX-treated rats displayed focal necrotic cell death, congestion-hemorrhage and myocytolisis, effects that were reduced with the combined treatment with HA or CLZ. Also, HA and CLZ prevented the DOX-induced oxidative damage, decreasing the levels of TBARS (nmol/mg of tissue) in heart (20.6 ± 2.6 CLZ + DOX vs. 32.5 ± 1.3 DOX; $P < 0.05$) and preserved heart functionality evaluated by molecular imaging with ^{99m}Tc-Sestamibi. DOX produced severe histopathological alterations in the liver including focal necrosis and fibrosis, sinusoidal atrophy and edema. HA and CLZ markedly preserved hepatic tissue structure showing mild vacuolization. Accordingly, both compounds reduced DOX-induced enhanced-TBARS and cholesterol levels in rat liver. Dynamic hepatobiliary scintigraphy showed a reduced ^{99m}Tc-disida extraction with DOX administration (4 ± 1 vs 8 ± 3 min, $P < 0.05$), an effect that was blocked with the combined treatment with HA (9 ± 2 min). Similar cytoprotective effects were observed in *nude* mice.

We conclude that HA and CLZ exhibit chemoprotective effects against DOX-induced cytotoxic and oxidative damage in heart and liver and thus, they might improve the therapeutic index of this chemotherapy drug.

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INVOLVEMENT OF THE HISTAMINERGIC SYSTEM IN CENTRALLY-ACTING LEPTIN-EVOKED RESUSCITATING EFFECT IN HAEMORRHAGIC SHOCK IN RATS

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Leptin, acting centrally as a neuromodulator, induces the activation of the sympathetic nervous system, which may lead to a pressor action in normotensive animals. In haemorrhagic shock, leptin administered intracerebroventricularly (icv) evokes the resuscitating effect, with long-lasting rises in mean arterial pressure (MAP) and heart rate (HR), subsequent increase in peripheral blood flows, and a 100 % survival at 2 h. Since leptin is able to activate

histaminergic neurons, and centrally acting histamine induces the resuscitating effect, with the activation of the sympathetic nervous system, in the present study, we investigated an involvement of the histaminergic system in leptin-evoked cardiovascular effects in haemorrhagic shock. The model of irreversible haemorrhagic shock, with MAP decreased to and stabilised at 20–25 mmHg, has been used (4–6 animals per group). Leptin (20 μg) given at 5 min of critical hypotension evoked over 180 % increase in extracellular hypothalamic histamine concentration as measured 10 min after administration. Rises in MAP, HR and renal, mesenteric and hindquarters blood flows induced by leptin were inhibited by icv pre-treatment with histamine H_1 receptor antagonist chlorpheniramine (50 nmol, 5 min before leptin administration). In contrast, there was no effect of H_2–H_4 receptor antagonists ranitidine (25 nmol), VUF 5681 (25 nmol) and JNJ 10191584 (25 nmol), respectively. In conclusion, the histaminergic system is involved in centrally-acting leptin-induced resuscitating effect in haemorrhagic shock in rats.

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FURTHER CHARACTERIZATION OF HISTAMINE H_2 RECEPTOR OVEREXPRESSING MICE

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We have generated mice which overexpress the human histamine-H_2—receptor only in the heart (TG) and compared them with wild type littermates (WT).We have previously shown that in isolated preparations (atrium, perfused heart) or intact mice (echocardiography) histamine and dimaprit exert positive inotropic (PIE) and positive chronotropic effects (PCE) which were blocked by the H_2—antagonist cimetidine. We intended to further characterize pharmacologically the cardiac human H_2-receptor in these mice. We performed contraction studies in the organ bath.

The PIEs of dimaprit (1 μM) in electrically driven (1 Hz) isolated left atria of TG were reduced (by 23 %) by the PKA inhibitor H89 (50 μM, $n = 6$, $p < 0.05$)) or (by 24 %) by the inhibitor of the calcium calmodulin dependent protein kinase (Cam kinase, 50 μM) W87 ($n = 13$).

In right isolated atrial preparations from TG (5 from 6 preparation) but not WT (none from 6 preparations), dimaprit (1 μM) was able to induce arrhythmias ($p < 0.05$). These arrhythmias were blocked by additionally applied cimetidine (10 μM, 4 from 4 preparations).

The concentration response curves for histamine (0.1 nM to 1 μM) on force of contraction were shifted to higher concentrations of histamine ($n = 3$–6) in electrically driven left atrial preparations ($n = 3$–6) from TG after pretreatment with 100 μM histamine or dimaprit, which were subsequently washed out.

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HISTAMINE STIMULATES AMYLASE RELEASE IN NORMAL AND INFECTED RAT SUBMANDIBULAR GLANDS

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Salivary glands are exocrine glands that produce and secrete saliva into the mouth. The submandibular gland (SMG) is a serous/mucous gland that secretes substances such as mucin, amylase, proline-rich proteins and growth factors, among others. Amylase is one of the enzymes that participate in the protective, digestive and antibacterial functions of the saliva. Bacterial infections of the SMG and parotid glands are the most frequent cause of sialadenitis. The most common pathogens associated with acute gland infection are *Staphylococcus aureus* and anaerobic bacteria. The goal of the present work was to determine the role of histamine in amylase release from the normal rat SMG and in a sialadenitis model. The SMG infection model developed in our laboratory produces a classic non-specific acute inflammation, with a peak between 48 and 72 h post inoculation with *S. aureus*, when abscesses become apparent. We determined amylase secretion using the Bermfeld method in three-month old male Wistar rats. SMG slices from both normal and infected rats were incubated in the presence or absence of histamine (10^{-10}–10^{-5} M) plus pyrilamine (H_1 antagonist, PY); JNJ7771091 (H_4 antagonist, JNJ); U73122 (phospholipase C (PLC) inhibitor); L-NMMA (nitric oxide synthase (NOS) inhibitor); ODQ (soluble guanylate cyclase (GC) inhibitor) or A23187 (calcium ionophore). Histamine produced a concentration-dependent stimulation of total amylase activity for normal salivary glands (5.5 ± 0.5 vs. 9.2 ± 0.5, for basal and 10^{-6} M histamine, respectively, $p < 0.05$), an effect that was inhibited by PY but not by JNJ. U73122, L-MNNA and ODQ also blocked histamine-induced amylase release, while A23187 stimulated amylase secretion. In conclusion, histamine modulates amylase release in both normal and infected SMG by activation of H_1

receptors, activation of PLC, NOS and GC. This action is likely mediated by [Ca^{2+}].

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HISTIDINE AND HISTAMINE IN EUROPEAN WINES

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Wine, particularly red wine, is a beverage that contains significant amounts of histamine, which is thought to be the main cause of adverse reactions to wines. Many of the typical histamine-related symptoms can appear—without a true IgE-dependent allergic reaction—after wine consumption. Previously, it has been shown that wines (red, white wine, champagne) from different regions contain elevated concentrations of histamine. In this study we investigated the concentration of histidine and histamine in wines produced in southern Russia. The assessment was performed in nine different non-bottled barrel wines (red, white wine, Champagne and Jerez) by amino acid assay and fluorimetry following HPLC, respectively. It has been shown that all wines contain different levels of histidine as well as histamine. The concentration of histidine increased with time of fermentation and with the type of yeast employed. The highest concentration was determined in Jerez wines which was primarily formed after fermentation had ceased. After 6 months, the concentration of histidine in Jerez increased by 3–4-fold. The level of histamine in red wines (Cabernet and Merlot) was higher than in white wines (Chardonnay and Riesling). The concentration of histamine in two types of Jerez wines was strikingly increased (up to 2.8–6.5 mg/ml), possibly as a consequence of its production by yeasts during continuous fermentation. The high concentration of histamine may explain the appetizing activity of Jerez wines. Despite the elevated level of histamine and histidine in some types of wine, in a 1-month clinical study (with daily consumption of wines form 100 to 300 ml), we did not observe any clinical or immunological adverse effects of the wines with elevated histamine concentration.

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THE ROLE OF TECHNOLOGICAL FACTORS AND YEAST STRAINS ON THE CONCENTRATION OF HISTAMINE IN VARIOUS TYPES OF ALCOHOLIC DRINKS

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The concentration of histamine in various types of white and red table wines, liqueurs (including sherry), sparkling wines and champagnes was measured. Fermentation was varied by changing the duration time of biomass contact to the specific wine materials as well as the use of various strains of yeasts especially in the beer beverages. All the investigated alcoholic beverages were produced in the Russian Federation. Histamine concentrations were analyzed photometrically after HPLC-separation. An essential influence on histamine concentrations was observed due to the type of the yeast employed as well as other parameters, such as fermentation time. The highest concentrations of histamine were revealed in sherry produced by the "film" method (170–320 mg/l). Using the filmless methods up to 88 mg/l could be measured in beers especially when using "surface" and "bottom" fermentation methods where the concentration of histamine varied from 1.6 to 34 mg/l and from 35 to 58 mg/l, respectively. In champagne wines—made by the secondary fermentation method within the bottles—the concentration of histamine peaked at 32 mg/l and in sparkling wines produced by secondary fermentation in technological tanks (at two-triple use of the same yeast biomass) histamine levels ranged from 13 to 34 mg/l. In white and red table wines the concentration of histamine was much lower than in beer, champagne, sparkling wine and especially in sherry. It can be concluded that the concentrations of histamine—especially in wine—were highly dependent on the type of yeast and technological factors of the fermentation processe and production methods.

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INCREASING RADIOSENSITIVIY WITH HISTAMINE IN 1205 LU HUMAN MELANOMA CELLS

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Melanoma is a highly metastatic cancer, which is markedly resistant to conventional therapy and its global incidence continues to increase. The expression of histamine receptors in melanoma cell lines and a role for histamine in melanoma cell growth has been previously reported. In addition, the radioprotective potential of histamine on healthy tissue has been previously demonstrated. The aims of this work were to investigate the combinatorial effect of histamine and gamma radiation in vitro and in vivo on the radiobiological response of 1205Lu human melanoma cells and to explore the potential molecular mechanisms of the radiosensitizing action. For this purpose, we evaluated the response to gamma radiation (0 to 6 Gy). Cells were treated with histamine (10 μM) 24 h before irradiation and radiobiological parameters, including 2 Gy dose (D) surviving fraction (SF2 Gy), were obtained from the survival curves adjusted to the linear quadratic model [$SF = e^{-(\alpha D+\beta D2)}$]. Cell apoptosis was determined by the TUNEL assay and Annexin-V staining, while DNA damage was investigated through evaluating the levels of 8-hydroxy-2-deoxyguanosine and gamma-H2AX after 2 Gy dose. Antioxidant enzyme activity and reactive oxygen species (ROS) levels were measured spectrophotometrically and by flow cytometry, respectively. Results indicate that histamine increased radiosensitivity of 1205Lu cells (SF2 Gy 0.14 ± 0.03 vs. 0.34 ± 0.02 in untreated cells; α 1.06 ± 0.01 vs. 0.42 ± 0.07 in untreated cells, $P < 0.05$, T-test). Histamine enhanced radiation-induced oxidative DNA damage, apoptosis and senescence. It is important to highlight that histamine (1 mg/kg.day, subcutaneous administration) was able to potentiate in vivo the antitumoural effect of radiation (5 doses of 2 Gy), decreasing tumour size. We conclude that histamine increased the radiosensitivity of 1205Lu cells, suggesting that it could improve cancer radiotherapy for the treatment of melanoma.

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IONIZING RADIATION-INDUCED INVASIVE PHENOTYPE: HISTAMINE ACTIONS IN MCF-7 CELLS

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A great deal of evidence shows that ionizing radiation can enhance the migratory and invasive abilities of epithelial cancer cells through the acquisition of mesenchymal features and an associated invasive phenotype. We have previously demonstrated that histamine can block mesenchymal changes induced by 2 gray irradiation in pancreatic adenocarcinoma and triple negative breast cancer cells.

The aim of this work was to investigate further the role of histamine in modulating the ionizing radiation-induced invasive phenotype in the breast luminal cancer line, MCF-7.

Cells were cultured for 24, 48 or 110 h with or without different doses of histamine and irradiated or not with a dose of 2 gray of gamma radiation, which is comparable to that used in fractionation radiotherapy.

In non-irradiated cells, a concentration-dependent effect of histamine was detected. Histamine concentrations between 0.5 and 5 μM produced cells with spindle-shaped morphologies (observed by optical microscope) and a decrease in the expression of the epithelial marker, E-cadherin, by immunoblot. These concentrations of histamine also induced significant increases in the expression of mesenchymal markers, vimentin and slug, by western blot ($p < 0.05$) and in the expression of nuclear slug and beta-catenin by indirect immunofluorescence ($p < 0.05$), accompanied by a significant enhancement of migratory and invasive capacities as studied in transwell units ($p < 0.05$). However, histamine concentrations in excess of 20 μM did not induce mesenchymal changes in MCF-7 cells.

In irradiated cells, an invasive phenotype was induced as determined by the migration/invasion assays and the biomarkers analyzed above. Interestingly, histamine concentrations above 20 μM could reduce the manifestation of these mesenchymal features in irradiated cells ($p < 0.05$).

The identification of pharmacological agents that may regulate tumor cell proliferation and simultaneously control metastatic ability may be an effective way to increase the sensitivity of cancer cells and decrease side effects of radiotherapy.

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