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The European Histamine Research Society 45th Annual Meeting, May 11–14, 2016 Florence, Italy

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Meeting Report of the European Histamine Research Society

G. Sturman

This year's meeting was in Florence, Italy at the kind invitation of Professor Emanuela Masini. This is the fourth time that histaminologists have met in Florence and the second one that Emanuela has organised, the first being in 2007. The two previous Florence meetings had been organised by one of our Honorary Members, Pier Francesco Mannaioni and they took place in 1984 and 1975. Most of the delegates arrived on the Wednesday and this year there were 94 participants and 14 accompanying persons registered and they represented nearly 20 countries (mostly from Europe but also from North, South and Central America, Japan and Korea as well as some of the 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. The Council met as usual late afternoon. This year the meeting started formally on the Wednesday evening with the Opening Ceremony for the 45th meeting of our society in the Rettorato Lecture Hall of the University of Florence. We were welcomed by Professor Marco Bindi, Dean for National and International Research of the University of Florence, and Professor Alessandro Mugelli, Elected President of Italian Pharmacological Society. Our hostess, Professor Emanuela Masini and Paul Chazot, our President then talked about this meeting and paid tribute to Piero Mannaioni, an Honorary Member of the EHRS and now retired from Florence University. Then we listened to a lecture from Professor Donatella Lippi, Professor of History of Medicine at Florence University entitled "*Life and Death of the Medici Family*". She talked about this powerful Florentine family and the forensic work which has been undertaken on their remains. After this ceremony we then walked a short distance to the *"Giardino de' Semplici"* the Botanical Garden of the University which we wandered through before being taken to the University's Museum of Minerals and Stones. Then we returned to the Botanical Gardens for the Welcome Reception. Here we greeted old and new friends and exchanged news during the rest of the evening but also were able to marvel at the various plants on display in the Botanical Gardens—it was an excellent start to the meeting.

This year's meeting was held in the *"Maglio Cloister"*, which had been a convent for Dominican Nuns, but is now used by the Italian Army as their Medical School/Headquarters. On the Thursday morning, during the Opening Session we were formally welcomed by Emanuela who thanked Director of the Maglio Cloister for the use of this venue. Also thanks were given to all the various helpers. She also reported that there had been 79 abstracts submitted giving us 5 oral sessions, 5 poster sessions and also 5 invited speakers. Then we were welcomed again by our President, Paul Chazot, who then gave out the student bursaries certificates and € 500 for each of the 13 student members. The El-Sayed Assem family very generously sponsored two students while the rest were sponsored by our society.

Then we had the Honorary Membership ceremony where Associate Professor Anita Sydbom of Karolinska Institute, Sweden was presented with her official certificate beautifully written in Latin and sporting the society's official seal. The oration was given by Madeleine Ennis and Gill Sturman who spoke about many facets which Anita has given to the society and this included also the musical aspect. Thus it was fitting that this oration ended with a song dedicated to Anita. Anita then thanked the society for this honour. The scientific programme began with the first plenary lecture being the G. B. West lecture which was given by Patrizio Blandina of Florence University and was entitled *"The role of histamine in the memory of emotionally-salient experiences"*. He was introduced by Pertti Panula, Finland. At the end of this very interesting lecture, Patrizio was presented with a copy of GB West's autobiography—*"A Handful of Luck"*. This lecture was an excellent start to the first symposium which was on histamine and the Central Nervous System (CNS). Just before lunch we had the second plenary lecture and this was introduced by Madeleine Ennis. Professor Hiroyuki Fukui of Tokushima University, Japan gave a very enlightening lecture on *"Histamine H_1 receptor gene as a sensitive gene of nasal hypersensitivity and clinical significance of antihistamines"*.

In the afternoon there were two poster sessions; both on histamine and the CNS and also oral presentations on histamine and inflammatory responses before the third plenary lecture which was delivered by Holger Stark, Dusseldorf University, Germany and introduced by Astrid Sasse, Ireland. Holger talked about *"The development of histamine H_3 receptor antagonists from the bench to the bedside and back again"*. Then we went for a very interesting visit to the Cloister of Basilica della Santissima Annunziata (Basilica of the Most Holy Annunciation) which is a Renaissance-styled basilica and the Chapel of the Artists. The buildings were founded in 1250 by the seven original members of the Servite Order and many

artists are buried in its vault. The Florentine brides traditionally visit this shrine depicting the Annunication to leave their bouquets. Here we were had a very informative guided tour and saw a number of paintings being restored.

Friday started with another plenary lecture and this one was given by Ivan Izquierdo, Pontificial Catholic University of Porto Alegre, Brazil. Beatrice Passani gave the introduction. Ivan told us about "*The involvement of histamine in memory consolidation*". This was followed by the third symposium which was on histamine receptors. After coffee, we had a Round Table on the *'New Insights in Histamine and Cells of Inflammation*'. This was chaired by Ekaterini Tiligada, Greece and Carlo Riccardi, Italy and part of this round table was an invited lecture given by Francesca Levi Schaffer, The Hebrew University of Jerusalem, Israel on "*Mast cells the masterminds of allergic inflammation: What is now out there to inhibit their function?*" The morning ended with another poster session on Histamine Ligands in Metabolism and Adverse Effects.

After this session, we ate our packed lunches before boarding coaches for our trip to Lucca. We drove northwestwards from Florence to the Tuscan walled town of Lucca. Although we were driven on motorways to Lucca, we were able to see a little of the Tuscan countryside. Our first impression of Lucca was its huge wall which circles the old town. Here we were given a guided walking tour of Lucca. Initially we were taken for a short walk on the wall which sports a road of over 4 km in length. Then we descended from the wall and went into the old town. We were taken to Lucca's Cathedral (Duomo di San Martino). The Duomo contains the most precious relics of Lucca; the Holy Face of Lucca (a cedarwood crucifix), an image of Christ as well as the sculpture of the tomb of Ilaria del Carretto by Iacopo della Quercia. We heard about and saw many of the churches in Lucca and also visited Piazza the Anfiteatre, which has been restored/built in the ovoid shape of a Roman Amphitheatre making it into a modern square. Finally we were taken to the Real Collegium, the old convent of Saint Frediano Basilica, which under the Grand Duke Ferdinand in the Eighteen century became a Lyceum, where we had our dinner.

The following day the meeting started with presentations from 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 a very difficult task for the judges to differentiate between these nine excellent presentations. The winner was Roberta Fabbri (Florence, Italy) for her presentation entitled "*Brain histamine modulates inhibitory-avoidance memory recall by activating H_1 receptors in hippocampal CA1*" and the runner-up was Reggie Bosma (Amsterdam, The Netherlands) for her presentation entitled *"Residence time of antihistamines and its effect on signaling of the histamine H_1 receptor"*. The rest of the finalists were given Highly Commended Certificates. During the coffee break we had a group photo taken and then during the lunch, the Early Stage Researchers met with two of the Council Members (Arianna Rosa and Vanina Medina) to talk to about their ideas/wishes for the society. In the afternoon the final poster presentation sessions which were on Inflammation and New Therapeutic options took place. The final symposium of the meeting was on "Histamine and Future Clinical Development".

After this session there was a further viewing of the best 7 posters as judged by the poster jury (Paul Chazot, Jules Heuberger, Jerzy Jochem, Linda Kay, Cecilia Lanzi, Vanina Medina, Gustavo Provensi, Anwar Rayan, Arianna Rosa and Francisca Sánchez-Jiménez) who had been working very hard during the meeting and as usual had a difficult task in identifying winning posters for the poster competition. Eventually first prize was given to Kristine Rossbach et al., from Hannover, Germany with the poster entitled *"Anti-inflammatory effect of a combined treatment with an H_1R antagonist and an H_4R antagonist in a mouse model of atopic dermatitis"*, second to Sabrina Rahman et al., from Amsterdam, The Netherlands with the poster entitled *"Differences in β-arrestin2 recruitment by human H_3R isoforms as determined by Enzyme Fragment Complementation"* and third prize jointly went to Tadaho Nakamura et al., from Sendai, Japan with the poster entitled "*Electroencephalogram of H_1KO mice under isoflurane anesthesia"* and to Katrin Schaper et al., from Hannover, Germany with the poster entitled *"Impact of histamine on type 2 immune response"*. The authors of the three other posters short listed were given Highly Commended Certificates.

After this we held our General Assembly where the main issues discussed were the updating of our statutes and the website. This was followed by our traditional Farewell Dinner which took place in the prestigious historical Palazzo Budini Gattai in the heart of Florence. During the live concert by Les Moquettes and the Dirty Boots, the Award Ceremony with certificates and prizes were 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 'Oh Susanna') as a big thank you to Emanuela 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 the Italian histaminologists, especially Emanuela Masini and Beatrice Passani for the excellent meeting. The next meeting will be held in Amsterdam, The Netherlands (10–13 May, 2017) and will be a joint meeting with the Japanese Histamine Research Society. It will be organised by Rob Leurs and colleagues.

Participants of the 2016 meeting of the EHRS.

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Honorary Memberships in the European Histamine Research Society

M. Ennis, 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 14 current Honorary Members of the Society; Madeleine Ennis, Agnieszka Fogel, Robin Ganellin, Helmut Haas, Zsuzsanna Husti, Piero Mannaioni, Emanuela Masini, Bruno Mondovi, Fred Pearce, Jean-Charles Schwartz, Henk Timmerman, Ingrid Olhagen-Uvnäs, Takehiko Watanabe and Jean West. Former (now sadly deceased) 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 contributed significantly to the EHRS over the years and she is Assistant Professor Anita Sydbom of the Karolinska Institute, Sweden.

The oration for Anita was given by two of her closest EHRS friends; Madeleine Ennis and Gill Sturman. Anita studied Chemistry, Zoology and Microbiology at the University of Stockholm, graduating with a Bachelor of Science in December 1966. She then began her life-long association with the Karolinska Institute working with her scientific father and mentor, the late Professor Börje Uvnäs. This was a very fruitful collaboration and she obtained her PhD in 1982. Her thesis was entitled: Anaphylactic histamine release from isolated rat mast cells: Pharmacological and methodological studies and several honorary members of the EHRS wrote to her congratulating her on this fine piece of work. The slight delay was caused by the birth of three of her four children during her PhD studies! She continued her research in the Karolinska Institute, starting another long-term collaboration with Sven-Erik Dahlén and also taught pharmacology. In 1989, to further her career she started studying medicine and discovered another passion, helping patients. She has continued with this dual role of working as a researcher and a medical doctor. However, Anita never makes things easy; her clinical work is in Funäsdalen a mere 568 km north of Stockholm!

Anita has been a member of the EHRS Member since 1979 and participated in 34 meetings and presenting her research, only missing a very few meetings due to illness or pregnancy. She has held many positions of responsibility within the society: 1983–1984, 1989 Vice National Secretary, 2003–2006 Council member, 2006–2012 President of the EHRS, 2012–2013 Past President of the EHRS and she took over as President in 2013/2014 due to the serious illness of the President, Paul Chazot. In 2008, she organized the Stockholm EHRS meeting. Anita is also a great poet and singer. She has been updating the EHRS anthem annually since 1990 and since 1988 has written a special song for each of the meetings. In 2008, we wrote one for her as she did not want to write one for herself! Many of the younger members will know that she is a great photographer making sure that the society has a photo of every member attending the meetings. Anita is a fantastic supporter and ambassador for the society and rightly deserves being elected an Honorary Member of the European Histamine Research Society.

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The presentation of Honorary Membership to Associate Professor Anita Sydbom by Paul Chazot, President of the EHRS with Professor Emauela Masini, hostess for this meeting.

Immunological aspects of histamine

HISTAMINE H_1 RECEPTOR GENE AS A SENSITIVE GENE OF NASAL HYPERSENSITIVITY AND CLINICAL SIGNIFICANCE OF ANTIHISTAMINES

H. Fukui

Nasal hypersensitivity is a representative incurable disease. Antihistamines are the first choice for the therapy of this disease. Antihistamines target histamine H_1 receptor although the mechanism of antihistamines to alleviate the symptoms remains to be elucidated. The mechanism of histamine H_1 receptor-mediated H_1 receptor up-regulation was recently discovered. The significant correlation between nasal symptom scores and level of H_1 receptor mRNA in nasal mucosa was also observed in patients with pollinosis, suggesting that the H_1 receptor gene is an allergic disease-sensitive gene. The H_1 receptor up-regulation was induced by the activation of protein kinase C-delta (PKCδ) and downstream elevation of H_1 receptor gene expression.

(−)Maackiain isolated from *Kujin*, an anti-allergic Kampo medicine found to target heat shock protein 90 (hsp90), dissociated Hsp90-PKCδ complex and suppressed H_1 receptor gene expression. Alleviation by (−)maackiain and Hsp90 inhibitors of hypersensitivity symptoms also suggests that the H_1 receptor gene is an allergic disease-sensitive gene. The elucidated pathological mechanism is thought to be a good tool for the establishment of nasal hypersensitivity therapy with highest satisfaction.

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MAST CELLS THE MASTERMINDS OF ALLERGIC INFLAMMATION: WHAT IS NEW OUT THERE TO INHIBIT THEIR FUNCTION?

F. Levi-Schaffer, R. Singh Gangwar, M. Seaf, L. Karra, N. Landolina

Mast cells (MCs) are the primum movens of the allergic response. Moreover we have defined a pro-inflammatory crosstalk (the Allergic Effector Unit, AEU) between MCs and the infiltrating eosinophils (Eos) that stresses the role of MC role in late phase and chronic development of allergic inflammation. Therefore, inhibition of MCs is a main pharmacological target to limit allergy and possibly other MC driven diseases. We aimed to define activating (AR) and inhibitory (IR) receptors on MCs as druggable options for the treatment of allergic diseases. We used human cord blood derived MCs (CBMC) and mouse bone marrow derived MCs (BMMC). Mice models were atopic dermatitis (AD), allergic asthma (AA), allergic rhinitis (AR) and allergic (AP) and *S. aureus* enterotoxin B (SEB) induced peritonitis (SEB-P). FC, WB, RT-PCR, various functional assays for in vitro receptor studies were used. In the in vivo models, inflammatory infiltrate and specific markers of the disease were followed. We defined CD48, a GPI CD2 family receptor, as a main AR expressed on MCs that can be activated by the binding to its high affinity ligand 2B4 as expressed on Eos and by *S. aureus/SEB* a known infecting pathogen of allergic diseases and by specific mAbs. Its blocking or absence (CD48−/− BMMC) decreased significantly MCs activation. Similarly AD or AP or SEB-P disease markers were significantly reduced in CD48−/− mice. We also described CD300a and Siglec-7 as main IRs on MCs. The activation of these receptors carried out by specific mAbs downregulated the in vitro MCs functions by a distinct ITIM-mediated signal transduction pathway. Moreover in a CD300a−/− model of AP inflammation was significantly enhanced. Blocking of CD48 on MCs can be a therapeutic option in selected AD and asthmatic patients. Siglec-7 and CD300a activation by mAbs can be a more general option to inhibit MC activation in any allergic and non-allergic disease in which MCs have an activated phenotype.

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TREATMENT WITH ANTIHISTAMINES IN COMBINATION WITH SUPLATAST TOSILATE MARKEDLY ALLEVIATED NASAL SYMPTOMS IN TOLUENE-2,4-DIISOCYANATE-SENSITIZED RATS

T. Wakugawa, H. Mizuguchi, T. Kadota, A. Sawada, T. Kominami, Y. Kitamura, N. Takeda, H. Fukui

Antihistamines inhibit histamine signalling by blocking the histamine H_1 receptor (H_1R) or suppressing H_1R signalling as inverse agonists. The H_1R gene is upregulated in patients with pollinosis; its expression level is highly correlated with the nasal symptom severity. Thus, antihistamines that suppress H_1R gene expression are widely used as allergy treatments. However, long-term treatment with antihistamines does not completely resolve toluene-2,4-diisocyanate (TDI)-induced nasal symptoms, although it can decrease TDI-induced upregulation of H_1R gene expression to the basal level, which suggests that other types of signalling are responsible for the pathogenesis of the allergic symptoms. Here, we show that treatment with antihistamines and suplatast tosilate markedly alleviates nasal symptoms in TDI-sensitized rats. Suplatast (500 μM) suppressed ionomycin/phorbol-12-myristate-13-

acetate-induced upregulation of IL-2 gene expression in Jurkat cells (65.1 ± 6.7 %, N = 4, $P < 0.01$ 1-way ANOVA), in which calcineurin (CN)/Nuclear factor of activated T-cells (NFAT) signaling is known to be involved in IL-2 gene expression. Immunoblot analysis demonstrated that suplatast inhibited binding of NFAT to DNA (basal level). Furthermore, suplatast suppressed ionomycin-induced IL-9 mRNA upregulation in RBL-2H3 cells, in which CN/NFAT signalling is also involved. Data suggest that combined therapy of antihistamines and suplatast could be effective in alleviating nasal symptoms in allergic rhinitis. The mechanism underlying this therapy appears to be the suppression of both H_1R and CN/NFAT signalling by antihistamines and suplatast, respectively.

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HISTAMINE IN INFLAMMATION—THE INVOLVEMENT OF HISTAMINE H_4 RECEPTOR IN HUMAN EOSINOPHILS ADHESION TO ENDOTHELIUM

M. Grosicki, S. Chłopicki, K. Kieć-Kononowicz

Histamine is known to be involved in inflammatory responses that include cellular chemotaxis, migration and trafficking. Nevertheless, the relative role of histamine and histamine receptors in the mechanisms of leukocyte adhesion to endothelial cells remains elusive. Therefore, the aim of this study was to examine the effect of selective histamine receptors ligands on eosinophil adhesion to the endothelium. Highly purified eosinophils (98 %) were isolated from the human peripheral blood. Their viability and functionality were validated in several tests prior to the experiments. Eosinophil adhesion to endothelium cells was evaluated during eosinophil co-culture with human Ea.hy.926 endothelium cell lines, under static conditions. In adhesion assays, the cells were exposed to: fMLP, histamine, and histamine receptor ligands that included: H_1 receptor antagonist/inverse agonist—diphenhydramine and mepyramine, H_2 receptor antagonists—famotidine and ranitidine, H_3 receptor antagonist—pitolisant, H_4 receptor antagonists—JNJ7777120 and H_3/H_4 antagonist—thioperamide. Both histamine and fMLP significantly ($P < 0.001$) increased the number of eosinophils that adhere to endothelium, in dose-dependent manner (with EC_{50} of 1.36 and 1.42 μM respectively). Among the selected histamine receptors ligands only JNJ7777120 and thioperamide had direct effect on eosinophil adhesion to endothelial cells, decreasing the number of adherent cells in presence of histamine (with IC_{50} value of 0.7 and 1.9 nM respectively). The H_4 receptors antagonists themselves were ineffective in eosinophil adhesion. No additional effect from histamine H_1 and H_2 receptor ligands was detected when exposed to high histamine concentrations. 4-methylhistamine, the selective histamine H_4 receptor agonist, increased the number of adherent cells with similar effectives to histamine (EC_{50} of 0.63 μM). These data demonstrate that histamine H_4 receptor plays a dominant role in histamine-induced eosinophil adhesion to the endothelium.

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HISTAMINE-GENERATING hdcA+ L. REUTERI SUPPRESSES INFLAMMATION AND REGULATES MYELOPOIESIS IN MOUSE MODELS

B. P. Ganesh, A. Hall, M. Whary, J. G. Fox, J. Versalovic

Probiotics may beneficially affect the disease course of patients with chronic immune-mediated disorders like inflammatory bowel disease and colorectal cancer (CRC) via modulation of host immune responses. A recent pangenomic study showed that human-derived clade II *L. reuteri* strains contained a complete chromosomal histidine decarboxylase (*hdc*) gene cluster (genes *hdcA*, *hdcB*, *hdcP*) and have the genetic capacity to convert histidine to histamine. In our lab we found *L. reuteri* 6475 (clade II) derived-histamine suppressed TNF production by human myeloid cells. In addition, we also showed that administration of *L. reuteri* to HDC deficient mice bearing inflammation-associated colon cancer (IaCC) showed suppression of inflammatory cytokines (mainly IL-6, IL-22, IL-1α and TNF). Apart from suppression of the inflammatory response, CD11b+ Gr-1+ immature myeloid cell (IMCs) populations were reduced in the spleen and bone marrow of *hdcA+ L. reuteri* administered to HDC KO mice bearing IaCC compared to HDC KO IaCC mice with *hdcA− L. reuteri*. These changes correlated with reduced tumor numbers in HDC KO mice. However, *L. reuteri* lacking histidine decarboxylase (hdcA mutant) were unable to produce histamine and did not protect HDC KO mice from IaCC. However, *L. reuteri* 6475-derived histamine and its ability to suppress inflammation and reduce IaCC are not well understood. We therefore hypothesized that *L. reuteri* derived-histamine down-regulates histamine

receptor 1(H_1R) and stimulates H_2R, thereby suppressing inflammation. We used Swiss-Webster WT and BALB/c WT germ-free mice mono-associated with *L. reuteri* wild-type or the *L. reuteri hdcA* mutant strain. Our results showed significant reduction of IL-6 expression in the GF mice receiving *L. reuteri* WT and *hdcA* mutant strains, $n = 10$ (5 males and 5 females each). In addition, we found that both WT and mutant strains can produce diacylglycerol kinase (dagK), inhibiting DAG signaling downstream and potentially blocking H_1R downstream signaling. Moreover, we showed that IL-6 is not suppressed in dagK mutant *L. reuteri* colonized GF mice. We also observed that dagK is a secretory protein by *L. reuteri* 6475 and was quantified using liquid chromatography mass spectrometry (LC–MS/MS) analysis. In conclusion, *L. reuteri* 6475-derived histamine induced H_2R downstream signaling and suppressed inflammation by inhibiting H_1R downstream signaling via dagK secretory enzyme in the host intestine.

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HISTAMINE REGULATES THE ACTIN CYTOSKELETON IN HUMAN TLR4-ACTIVATED mDCs TUNING CD4$^+$ T LYMPHOCYTE RESPONSES

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Histamine differentially regulates the polarizing ability of Dendritic Cells (DCs) after stimulation through Toll-like receptors (TLRs), although the mechanisms are not fully understood. TLRs are a class of receptors critical in host defense that specifically detect pathogen-associated molecular patterns and their action has been associated with loss of tolerance and pathogenesis of chronic inflammation, autoimmunity and allergy. In this study we investigated the effects of histamine (10 μM; for 6 days) on the innate immune reaction during the response of human monocyte-derived DCs (mDCs; $n = 5$) to different TLR stimuli: LPS, specific for TLR4 and Pam3Cys, specific for the heterodimer molecule TLR1/TLR2. We investigated mDC phenotype, cytokine production, and Th0 stimulatory and polarizing ability, together with analysis of the cytoskeleton of mDCs by confocal microscopy, and RT-PCR expression of Rac1/CdC42 Rho GTPases, responsible for actin remodeling. Our results show that histamine selectively modifies actin cytoskeletal organization induced by TLR4, but not TLR2, and this correlates with increased IL-4 production by primed T cells ($p < 0.05$). We also demonstrate that histamine-induced cytoskeleton organization is mediated by down-regulation of small Rho GTPase CdC42 and the protein target PAK1, but not by down modulation of Rac1. Independently of actin remodeling, histamine down-regulates IL12p70 and CXCL10 production in mDCs after TLR2 and TLR4 stimulation. We also observed a trend of IL-10 up-regulation that did not reach statistical significance. In conclusion, our data show that histamine differently shapes mDC TLR4 and TLR2 responses and we propose that, besides antigen presentation, costimulatory molecules and CKs milieu, this effect is also mediated by cytoskeletal rearrangements.

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HISTAMINE H_2 RECEPTOR STIMULATION UP-REGULATES TH2 CHEMOKINE CCL17 PRODUCTION IN HUMAN M2 MACROPHAGES

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Histamine receptors are possible therapeutical target structures for the treatment of atopic dermatitis. In allergic skin diseases such as atopic dermatitis, macrophages are attracted into tissue and exposed mainly to Th2 cytokines and also to histamine which is released in the skin during allergic reactions. In this study, we investigated the role of histamine on human monocyte derived M2 macrophages differentiated in the presence of M-CSF and activated with IL-4 or IL-13. H_1R- H_2R- and H_4R mRNA expressions were measured on fully differentiated and IL-4 activated monocyte derived M2 macrophages by quantitative PCR. We observed that the activation with IL-4 led to a significant up-regulation of the H_2R and H_4R at mRNA level ($p < 0.05$) in M2 macrophages. The activation of M2 macrophages with IL-4 or IL-13 resulted in higher expression levels of the Th2 cell attracting chemokines CCL22 and CCL17 at mRNA- and protein level. Interestingly, stimulation with histamine led to a significant more than two fold further up-regulation of CCL17 expression ($p < 0.01$) whereas the expression of CCL22 was not affected by histamine. To show which histamine receptor was responsible for the up-regulation of CCL17, we stimulated the H_1R, H_2R and H_4R on human IL-4 or IL-13 activated M2 macrophages. Of note, we observed that the effect was attributed to the H_2R. The stimulation of the H_2R with the selective H_2R agonist amthamine and 4-methylhistamine (H_2R/H_4R agonist) led to a significant up-regulation of the CCL17 expression at mRNA- and protein level ($p < 0.01$) in IL-4 or IL-13 activated M2

macrophages which could convincingly be blocked by preincubation with the specific H_2R antagonist ranitidine. In summary we show a new function of the H_2R by up-regulating the Th2 attracting chemokine CCL17 in human M2 macrophages which might lead to a pronounced attraction of CCR4 expressing Th2 cells into the site of inflammation and provide evidence for a role of histamine to support a Th2 dominated milieu. This might have an impact on the course of atopic dermatitis and for the treatment of the disease.

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NEW INSIGHTS INTO THE INTRACELLULAR CONTROL OF IgE-DEPENDENT HISTAMINE RELEASE FROM HUMAN BASOPHILS

B. F. Gibbs

Human basophils rapidly release histamine and other inflammatory and immunomodulatory mediators upon high-affinity IgE-receptor crosslinking and thereby play a crucial role in the pathogenesis of allergic diseases. While the presence of allergen-specific IgE on these cells is an important determinant of basophil-mediated allergic responses, it does not necessarily predict the severity of clinical symptoms of allergy per se. The aim of this study was to shed light on intracellular signals which are associated with the control of human basophil reactivity. Basophils were obtained from buffy coats purchased from the NHS Blood and Transplant service (following research ethics approval; REC 12/WM/0319) and highly purified (to over 90 % purity) by negative selection and magnetic cell sorting. Cells were incubated with IgE-dependent triggers, together with unstimulated controls, for varying periods after which histamine releases as well as intracellular signaling protein expressions were assessed by spectrofluorometric autoanalysis and Western blotting, respectively. We observed that constitutive SHIP-1 phosphorylation (but not total SHIP-1 or Syk expressions) in unstimulated basophil preparations correlated closely with maximum basophil responses in terms of histamine release to anti-IgE. Moreover, there was a striking inverse correlation between basophil responsiveness to IgE-dependent stimulation and expression of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2), a protein which controls intracellular calcium mobilization. These results clearly show that early excitatory or inhibitory signal transduction pathways are not the only mechanisms that determine the magnitude of IgE-dependent histamine release in basophils but that regulators of intracellular calcium mobilization also have critical input in allergic effector cell reactivity. Upregulation of SERCA2 could therefore be considered as a novel therapeutic approach in allergic effector cell-mediated diseases.

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NEUROINFLAMMATORY PATHWAY: ROLE OF NEURONAL AND NON-NEURONAL CELLS IN THE PNS AND CNS

S. Cuzzocrea

Neuroinflammation represents the response of the CNS to altered homeostasis. It is primarily mediated by the contribution of one/two cell systems: glia, lymphocytes, monocytes and macrophages of the hematopoietic system. Neuroinflammation can be triggered by classical factors such as infection, autoimmunity and toxins but also by toxic stimuli or psychological stress. In the CNS and PNS, glia and neurons, together with endothelial cells of the microvasculature, release cytokines and express their cognate receptors. The physiological functions of cytokines include neurite outgrowth, neurogenesis, neuronal survival and synaptic pruning during brain development. Moreover, they regulate the strength of synaptic transmission and synaptic plasticity. On the basis of current knowledge, a novel therapeutic approach to the treatment of neuronal diseases will be considered. Preclinical models of trauma or neurodegenerative diseases are employed to study the network involved in neuroinflammatory mechanisms. The complexity of neuroinflammatory and neurodegenerative diseases has hampered the identification of pharmacological agents able to ameliorate outcomes after damage. In this context, a new formulation composed of co-ultramicronized palmitoylethanolamide (PEA, an endogenous fatty acid amide signaling molecule) together with the flavonoid luteolin (Lut, co-ultraPEALut) has been employed. Due to the complex nature of the injury the best approach is provided by a combination of many therapeutic strategies to counteract the different aspects of the damage. Neuroprotection pertains to the preservation of the spared neurons and their processes following the injury. Neuroregeneration aims to modulate the lesion site

environment to promote axonal regrowth. Neurorehabilitation has demonstrated beneficial effects at cellular and molecular levels.

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CHARACTERIZATION OF THE ISOFORM OF CYCLOOXYGENASE THAT MEDIATES THE GENERATION OF PROSTAGLANDIN D2 FROM HUMAN LUNG MAST CELLS

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Mast cells are known to be the principal source of histamine in the body but they are also a very rich source of prostaglandin D2 (PGD2). Our previous studies have shown that activation of human lung mast cells by either anti-IgE or the growth factor, Stem Cell Factor (SCF), leads to the generation of high levels of PGD2. The enzyme cyclooxygenase (COX) is central to the generation of PGD2. COX generates intermediates from arachidonic acid that are then acted on by PGD2 synthase. COX exists as two main isoforms COX-1 and COX-2. The aim of this study was to identify which COX isoform is responsible for PGD2 generation from human lung mast cells. Mast cells were isolated from human lung tissue and purified by flotation over Percoll and immunomagnetic bead separation (85-98 % purity). The cells were activated with SCF (100 ng/ml) or anti-IgE (2 μg/ml) and the release of PGD2, histamine, and cysteinyl-leukotrienes was assessed by spectrofluorometry and ELISA. The effects of COX inhibitors, indomethacin (non-selective), FR122047 (COX-1 selective) and celecoxib (COX-2 selective) on mediator release were determined. Purified mast cells were solubilised and used to determine the expression of COX-1 and COX-2 by RT-PCR and Western blotting. Both SCF and anti-IgE were effective drivers of PGD2 generation from mast cells (n = 20). Indomethacin and FR122047 (0.1 μM) completely abrogated ($P < 0.05$, n = 4) the production of PGD2 from lung mast cells activated by SCF or anti-IgE while celecoxib (0.1 μM) was ineffective ($P > 0.05$, n = 4). None of these COX inhibitors affected the stimulated generation of either histamine or leukotrienes. Western blotting studies showed that COX-1 was strongly expressed in all mast cell preparations while the expression of COX-2 was, at best, weak (n = 4). These findings indicate that COX-1 is the principal isoform involved in PGD2 generation from human lung mast cells.

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L-ASPARAGINASE-INDUCED ALLERGY IN MICE: EFFECTS OF CONCOMITANT DRUGS AND ANTI-IgE ANTIBODY

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L-Asparaginase (L-Asp) derived from *E. coli* is one of the essential drugs for acute lymphoblastic leukemia. Native L-Asp often causes allergies, including anaphylaxis. At present, there is neither suitable therapy for L-Asp allergy nor alternative L-Asp in Japan. Moreover, there is no suitable animal model of L-Asp allergy. In this study, we induced type I allergy to *E. coli* L-Asp in mice and the effect of anti-IgE antibody was evaluated. In addition, we investigated the effects of concomitant drugs on this model using methotrexate (MTX) and cyclophosphamide (CY) which reportedly impair the functions of Th17 and Treg cells, respectively. Male BALB/c mice (7 week old) were i.p. injected L-Asp with $Al(OH)_3$ gel on days 0 and 14. The right ears of the mice were locally sensitized on days 17, 20, 23 by i.d. injection of L-Asp. Antigen challenge was carried out on day 26 by i.d. injection of L-Asp. Methotrexate (MTX) and cyclophosphamide (CY) were i.p. administrated at day –2 and day 12. On day 25, either anti-IgE Ab or control IgG was i.p. administered to the sensitized mice. L-Asp challenge immediately induced ear edema, reaching a peak at 1 h after stimulation, which subsided within 24 h. Microscopic observations showed eosoniphil infiltration (H&E staining; 0.5 ± 0.2 cells/HPF in control ears and 10.4 ± 1.0 cells/HPF in challenged ears, $P < 0.001$, n = 3) and degranulation of mast cells (toluidine blue staining; 8.4 ± 0.9 % in control ears and 47.6 ± 5.8 % in challenged ear, $P < 0.001$, n = 3). Such changes due to L-Asp challenge were inhibited by pretreatment with anti-IgE antibody (BD Bioscience, clone R35-92, which specifically reacts with mouse IgE of Igh-C[a] and Igh-C[b] haplotypes). Cyclophosphamide, but not methotrexate, enhanced L-Asp-induced ear edema. This augmentation was also inhibited by a pretreatment with anti-IgE antibody. From these results, it was concluded that (1) non-anaphylactogenic neutralizing antibody of IgE, such as omalizumab, can be a candidate drug for the treatment of L-Asp allergy, and (2) CY-induced suppression of Treg function may enhance Th2 responses so as to augment L-Asp allergy. Further investigations for the detection and avoidance of L-Asp allergy are under the way.

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ANTI-INFLAMMATORY EFFECT OF A COMBINED TREATMENT WITH AN H_1R ANTAGONIST AND AN H_4R ANTAGONIST IN A MOUSE MODEL OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic relapsing allergic skin disorder characterized by skin lesions, pruritus and elevated serum levels of allergen specific IgE. In a mouse model of AD, the ovalbumin (OVA)—induced model, H_4R knockout mice showed a clear amelioration of skin lesions compared to wild type mice. However, neither H_4R antagonists nor H_1R antagonists exhibit distinct anti-inflammatory effects in the OVA model. This study was performed to test the anti-inflammatory potential of a combined treatment with an H_1R antagonist (mepyramine) and an H_4R antagonist (JNJ39758979; JNJ) in the OVA—induced AD model. Mice were treated systemically during the challenge phase with a combination of JNJ and mepyramine (both 20 mg/kg twice daily). AD-like lesions were induced by repeated epicutaneous application of OVA. Severity of dermatitis was assessed by standardized skin score. As an indicator of pruritus, scratching behavior of mice was determined. Mice were sacrificed after the second challenge and samples were taken for further analysis. Mice treated with JNJ and mepyramine showed a clear amelioration of skin lesions, with a diminished influx of inflammatory cells and a reduced epidermal hyperproliferation compared to vehicle-treated mice. Scratching behavior was significantly reduced in mice treated with JNJ and mepyramine. Moreover, mice had a reduced amount of OVA-specific IgE in serum and a reduced number of splenocytes and lymph node cells. The cellular profile in skin-draining lymph nodes was not modulated. The level of OVA-induced IL-10 production of splenocytes was significantly higher in JNJ and mepyramine treated mice, whereas IL-4 and IL-6 secretion did not differ between the groups. Hardly any INF-γ was detectable in supernatants of splenocytes. JNJ and mepyramine-treated mice showed a reduced inflammatory response compared to vehicle treated mice. Thus the combined treatment with an H_1R and an H_4R antagonist might be a new therapeutic strategy to treat AD.

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IMPACT OF HISTAMINE ON TYPE 2 IMMUNE RESPONSE

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Type 2 immune responses are defined by the cytokines interleukin 4, (IL-4); IL-5, IL-9 and IL-13. While IL-5 serves mainly as stimulator of eosinophils, IL-13 also regulates cell-mediated immunity and thereby enhances mucus production and airway hyperreactivity. Allergic disorders, such as asthma or atopic dermatitis are associated with increased Th2 cytokines. Th2 cells are known to be the major source of IL-5 and IL-13, but also other cells like type 2 innate immune cells produce these cytokines. However, the role of histamine in the mechanisms of initiation and control of type 2 responses, especially the effect on the production of IL-5 and IL-13 is not fully understood. Therefore our aim was to investigate possible effects of histamine on IL-5 and IL-13 production of innate lymphoid cells as well as T helper cells. We therefore isolated PBMCs from healthy subjects and depleted CD3+ cells. CD127+ cells were further enriched by means of magnetic cell separation. On stimulation with IL-2 and IL-25 for 5 days CD3-CD127+ cells produced high amounts of IL-5 and IL-13, while the cytokine production of CD3+ cells was lower. CD3-CD127- cells and unstimulated CD3-CD127+ cells did not produce any detectable IL-5 and IL-13. Incubation of the cells with histamine during the 5 days of stimulation augmented the production of IL-5 and IL-13 in CD3-CD127+ cells significantly, but not in CD3+ cells. Thus, our results indicate that histamine foster a Th2 milieu by directly affecting IL-5 and IL-13 production in innate lymphoid cells.

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MODULATION OF ANTIGEN SPECIFIC T CELL PROLIFERATION BY HISTAMINE

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Dendritic cells (DCs) are potent antigen presenting cells and are able to induce proliferation of T cells. Both cell types are found in chronic inflammatory skin diseases. As histamine is also upregulated in lesions of inflammatory

skin diseases, we decided to investigate the role of histamine and its receptors on antigen specific T cell proliferation and interaction of T cells and DCs. Therefore, human peripheral blood mononuclear cells (PBMCs) of allergic donors were stimulated with allergens. According to the sensitization of the donor, Phl.p.5 (Phleum pratense), BetV1 (Betula pendula) or Der p2 (dermatophagoides pteronyssinus) were used at a concentration of 2.5 μg/ml. Stimulation was performed in the absence or presence of histamine or histamine receptor agonists and T cell proliferation was determined after 5 days using a thymidine incorporation assay. Only experiments with an increase of T-cell proliferation in response to allergen stimulation were assessed for the effect of histamine. In Der p2 stimulated PBMCs, stimulation with histamine and the histamine 4 receptor agonists 4-methylhistamine and ST1006 decreased T cell proliferation. This trend was also observed in PBMCs successfully stimulated with Phl.p.5 and BetV1 although the number of experiments is currently too low to draw firm conclusions. To preclude an interfering influence of endogenous histamine, we examined the supernatants of cell cultures by means of ELISA. Generally, histamine levels were below 4 ng/ml and 20 % of samples showed no detectable histamine with a detection limit of 1.3 ng/ml. Taken together our first results suggest that allergen specific T cell proliferation is modulated by the histamine 4 receptor.

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CARBONIC ANHYDRASE IX AND CYCLOOXYGENASE-2 INHIBITORS AS A NEW CLASS OF ANTI-INFLAMMATORY DRUGS

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The carbonic anhydrase (CA) family includes 16 catalytically active zinc metallo-enzymes that catalyze the reversible interconversion of carbon dioxide and water to bicarbonate and protons. CA inhibitors are a potential new class of antitumour agents; the selective inhibition of CAIX decreases tumour cell proliferation and induces apoptosis in human cancer cells. Moreover, sulfonamide CAIX inhibitors exhibit anti-inflammatory effects in rats with permanent middle cerebral artery occlusion. Macrophages are involved in inflammation, and histamine up-regulates the production of pro-inflammatory cytokines stimulated by LPS.

The aim of the present study was to investigate the pharmacological profile of a new class of drugs (1e, 2e, 3e, 6e), endowed with CAIX and COX inhibition activity.

RAW 264.7 macrophages were incubated for 18 h with LPS (1 μg/mL) and pre-treated or not with different concentrations of the studied drugs (10^{-7}–10^{-4} M). The prostaglandin E_2 (PGE_2) production was quantified with a commercial ELISA kit. On human platelet-rich plasma (PRP), the inhibition of platelet aggregation, induced by 10 μM ADP after 5' incubation, was studied, in presence or absence of the studied compounds. The concentration of TXA_2 was measured as TXB_2 production with an ELISA method on PRP after clotting.

Our results show that macrophages contain significant amounts of histamine (<0.008 pg/cell). PGE_2 production is lowered in LPS-stimulated RAW 264.7 cells treated with CAIX/COX inhibitors with a dose–response effect (Vehicle: 287.74 ± 11.3 pg/mL; 1e 10^{-5} M: 89.28 ± 7.65 pg/mL; ibuprofen 10^{-5} M: 116.5 ± 7.81 pg/mL). CAIX/COX inhibitors do not modify neither the inhibition of platelet aggregation in comparison with the reference molecules (baseline: 100 %; 1e 10^{-4} M: 28.97 %; ibuprofen 10^{-4} M: 26.71 %), nor the production of TXB_2 in comparison with the reference molecules (baseline: 218.25 ± 3.3 ng/mL; 1e 10^{-4} M: 100.72 ± 1.5 ng/mL; ibuprofen 10^{-4} M: 97.32 ± 4.3 ng/mL).

Our findings validate these molecules, endowed with a double COX-2 and CAIX inhibition, as interesting new anti-inflammatory drugs for a novel therapeutical approach to contrast cancer and inflammation.

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EXPERIMENTAL AUTOIMMUNE MYOCARDITIS (EAM) IN RATS AND THERAPEUTIC H_1-H_4 RECEPTOR INHIBITION

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Histamine plays an important role in immune and inflammatory responses. Produced by various cells and interacting with four G protein-coupled receptors, H_1-H_4, it can evoke different effects. Using rat model of autoimmune myocarditis, we investigated whether EAM progression could be influenced by chronic modulation of histamine receptor signalling. EAM was induced in male Lewis rats by immunization with porcine heart myosin in complete Freund's adjuvant (0.33 mg/each rear footpad), twice with one week apart. Compounds were applied daily for the first 2 weeks: for H_1R-Mepyramine (i.g. 20 mg/kg); H_2R-

Ranitidine (s.c.10 mg/kg); H_3R-Ciproxifan (s.c. 1 mg/kg), H_4R-ST 994 (antagonist), ST1012 (inverse agonist) (each by Alzet osmotic pump, 1 mg/kg). Quinapril (i.g. 20 mg/kg) was used as a reference drug. Echocardiographic examination was done before, and at 2 and 3 weeks of EAM. Plasma ceruloplasmin was used as an inflammation marker. Four weeks post myosin, all survivors were sacrificed by decapitation. Blood was collected from neck arteries, the hearts were excised, macroscopically evaluated and their weights recorded. The left and right atria and ventricles were dissected and immediately frozen on dry ice, heart apexes put into formalin for histology. The tissues were processed to measure hydroxyproline (spectrophotometry), histamine (ELISA) and transcripts for MCP-1 and TNFα (RT-PCR). Cardiac hypertrophy was seen in all EAM rats, however, it was lowest for ST994 treated ones. The means for heart weight to body weight ratio ranged from 0.62 to 0.99 % vs 0.30 % for intact rats. The % decreased fractional shortening, FS $= 12.8 \pm 2.87$ and left ventricular ejection fraction LVEF % 31.6 ± 6.31 show significant decrease in cardiac function by EAM progression. Both these parameters were higher in rats treated with histamine receptor antagonists, especially Mepyramine. However, left ventricle (LV) from Mepyramine-treated EAM rats had more copies of TNFα mRNA and MCP1 mRNA than LV from EAM rats, although the levels of both transcripts were the highest in LV of EAM-Ranitidine rats. Four weeks post EAM induction LV expressed a high degree of fibrosis, hydroxyproline concentration being 2–5 fold of control level. Histamine concentrations in LV were inversely correlated to hydroxyproline. All antihistaminics used, except ST1012 inverse agonist, efficiently reduced myocardial fibrosis as evidenced by LVED/S d, which was 0.03 cm for EAM vs 0.08 cm for ST 1012 while 0.12–0.19 cm for other receptor ligands. ST 994 inhibited inflammatory process at early stage (inflammatory cell infiltration) as indicated by unchanged LVEF and FS: 61 vs 67 % and 31 vs 32 %, respectively, for EAM + ST994 vs intact rats. There were great individual differences in disease progress and mortality, despite use of an inbred rat strain. The lowest mortality was seen in the EAM + ST994 group suggesting a novel use for H_4R-antagonists. This work was supported by the Grant No 2012/04/M/NZ4/00212 from National Science Centre, Cracov, Poland.

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Histamine in the central nervous system

THE ROLE OF HISTAMINE IN THE MEMORY OF EMOTIONALLY-SALIENT EXPERIENCES

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Phobias, panic attacks and PTSD are extremely common. Sitting at the heart of much fear is emotional memory—all the associations that one has between various stimuli and experiences and his emotional response to them. Memory is a multi-state process that includes acquisition, consolidation and retrieval. We report that the integrity of the brain histaminergic system is necessary for consolidation of long- term memory (LTM) of step-down inhibitory avoidance (IA), a task that explores emotional memory in animals. Histamine depletion impairs IA-LTM, and histamine infusion into in hippocampus or basolateral amygdala (BLA) restores LTM in histamine-depleted rats. The restoring effect in BLA occurs even when hippocampal activity was impaired. Cyclic adenosine monophosphate responsive-element-binding protein (CREB) phosphorylation correlates anatomically and temporally with histamine-induced memory recall. Thus, histamine neurotransmission appears critical to provide the brain with the plasticity necessary for IA memory through recruitment of alternative circuits that provide compensatory plasticity when one brain structure is compromised. We report that cerebral histamine depletion impairs also retrieval of IA in rats, and blunts retrieval-induced c-Fos activation and CREB phosphorylation in the hippocampus. Histamine infusion into the hippocampus restores IA retrieval in histamine-depleted rats by targeting brain H_1 receptors. Our study uncovers previously unidentified mechanisms involved in memory retrieval, and offers targets for developing novel pharmacotherapies to treat dysfunctional aversive memories, as well as to improve the efficacy of exposure psychotherapies. Memory determines the uniqueness of our personal history, and is decisive for each individual to survive and prosper. However, irrational fear is no longer useful and life-saving, and drugs decreasing painful emotional memories can help to get rid of pathological anxiety.

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HISTAMINE REGULATES MEMORY CONSOLIDATION

I. Izquierdo

The effect on memory consolidation of intracerebral microinfusions of histamine, its various H_1, H_2 and H_3 receptor agonists and antagonists and the histamine enhancer, SKF 94188 was recently studied in detail in various papers from our group. Immediate posttraining histamine given into the basolateral amygdala or the CA1 region of the hippocampus enhances memory consolidation of one-trial inhibitory avoidance in rats through H_2 and H_3 receptors respectively. Both effects are parallel and independent. In addition, it also enhances consolidation of the extinction of this task acting through H_2 receptors in hippocampus. Posttraining histamine also induces memory facilitation in the object recognition task, acting through both H_1 and H_2 receptors in hippocampal CA1 where, however, H_3 receptors have a depressant modulatory effect. The overall effect of histamine in this task is therefore complex. However, the findings show that histamine regulation is not limited to the memory consolidation of fear- or fear-related memories but is exerted also on that of more purely cognitive tasks. Interestingly, histamine acting through H_2 receptors in hippocampal CA1 reverses the deleterious effect of the exposure of rats to partial postnatal maternal deprivation on their memory consolidation of inhibitory avoidance when they reach adult age. This effect is shared by the cholinergic agents, donepezil and galantamine given orally and/or by physical exercise during young adulthood, suggesting that the chronic cognitive impairment caused by early maternal deprivation is a multifactorial phenomenon. This work was supported by the National Research Council of Brazil.

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BRAIN HISTAMINE MODULATES INHIBITORY-AVOIDANCE MEMORY RECALL BY ACTIVATING H_1 RECEPTORS IN HIPPOCAMPAL CA1

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Memory includes acquisition, consolidation and recall. Retrieval is the only measure of memory but little is known about its mechanisms. Histaminergic neurons are located in the tuberomamillary nucleus, send projections to the whole brain and regulate different types of memory. Here we study the involvement of brain histamine (HA) in inhibitory avoidance (IA) memory retrieval, which brain areas are involved, which HA receptor mediates HA action and the biochemical substrates required. Wistar male rats (3 months old; 300–330 g; 8–12 animals for each experimental group in inhibitory avoidance task and 4–5 animals for c-Fos protein immunostaining and Western-Blot

analysis of p-CREB) were implanted with cannulas in the lateral ventricle (LV), hippocampal CA1, basolateral amygdala (BLA) or ventromedial prefrontal cortex (vmpfCX). 24 h before or after inhibitory avoidance (IA)-training, when animals received a 2-s 0.5-mA scrambled foot shock, rats were infused i.c.v. with α-fluoromethyl-histidine (a blocker of histidine decarboxylase; α-FMH) or saline. 48 h after training rats underwent IA-retention test, and step-down latencies were evaluated. α-FMH rendered rats amnesic, independently of the time of infusion as shown by step-down latencies significantly shorter than those of controls. Application 10 min before the retention test of HA in CA1 of rats that had received α-FMH 24 h after training restored IA memory. HA was ineffective when given in the BLA or the vmpfCX. Intra-CA1 injections of a selective H_1 receptor agonists, but not of an H_2 agonist reinstated IA memory. The H_1 receptor antagonist pyrilamine disrupted IA memory retrieval in normal rats, thus suggesting a role of endogenous HA in IA memory retrieval. Neurochemical analysis showed that c-Fos immunopositive neurons were significantly less in the CA1 of 24 h after training α-FMH-treated rats, hence amnesic, compared with controls with normal IA memory. We found also reduced levels of pCREB, a crucial player in memory, in the CA1 24 h after training α-FMH-treated rats compared to controls. Targeting the histaminergic system modifies emotional memory retrieval, hence HA ligands may reduce dysfunctional aversive memories and improve the efficacy of exposure psychotherapies.

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HISTAMINE H_1 RECEPTOR OCCUPANCY IN HUMAN BRAIN MEASURED BY POSITRON EMISSION TOMOGRAPHY

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Histamine is considered a "bad guy" from an allergy point of view. However, histamine is functioning as a "good guy" in several other physiological aspects. In particular, histaminergic neurons form one of the most important neuronal systems that maintain and stimulate wakefulness. Histamine also functions as a bioprotector against various noxious and undesirable stimuli, for example, convulsion, nociception, drug sensitization, ischemic lesions, and stress. It may also be suggested that the activation of histaminergic neurons is important to maintain mental health. The guideline for allergic diseases, such as pollenosis and atopic dermatitis, recommends non-sedating antihistamines with low central nervous system (CNS) penetration to avoid suppressing histamine's CNS actions. Positron emission tomography (PET) is often used to evaluate the efficacy of CNS drugs by determining the drugs' neuronal receptor occupancy rate. While the blood concentration levels of a drug are frequently determined clinically, i.e., via therapeutic drug monitoring (TDM), CNS drugs do not necessarily show a correlation between their blood concentration levels and effect. We have reported on brain H_1 receptor occupancy measurements of antihistamines, antidepressants, and antipsychotics. The clinically most important contribution of PET studies on histamine H_1 occupancy in the brain of antihistamine-treated allergic patients is probably to the identification of the narrow therapeutic window of less than 20 % of systemic and topical treatments that permits optimal treatment without causing significant sedative side effects. Thus, these PET results may be useful for the development of optimal antihistamine dosing strategies. In the present review, the results of our previous studies on the significance of brain histamine H_1 receptor occupancy in human brain were summarized from the perspective of histamine function in the CNS.

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MEDIATORS OF INFLAMMATION IN THE ANTINOCICEPTIVE ACTION OF JNJ7777120 IN RATS

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Importance of histamine H_4 receptor in pain and mechanisms of action of its ligands are unknown. Its activity may be primary related to the anti-inflammatory effect. We analyzed antinociceptive and antinflammatory action of JNJ7777120 (J77) (25 mg/kg—i.p.) its interactions with COX and LOX inhibitors in acute nociception (AP), carrageenan-induced inflammation (CII) and adjuvant-induced arthritis (AIA) in LEW/CriCmed male rats (n = 7/group). Mann–Whitney U test was used to identify differences among groups with $p < 0.05$. Nociceptive thresholds (Randall-Sellito, Tail flick, Plantar test), edema, blood count, histamine, prostaglandin E2 metabolite (PGEM) and LTB4 levels in plasma, levels of MCP-1 in plasma and cerebrospinal fluid (CSF) and chemiluminescence of leukocytes were analyzed. In AP, J77 increased mechanical and thermal threshold and decreased concentration of MCP-1 in CSF and plasma. Indomethacin (3 mg/kg—i.p.) and celecoxib (3 mg/kg— i.p.) reduced J77 impact on the

MCP-1 level. Celecoxib decreased J77 activity towards mechanical but potentiated towards thermal stimulus. In CII, J77 reduced mechanical hyperantinociception, plasma concentration of LTB4 and chemiluminescence. Administration of celecoxib with J77 potentiated antinociception and reduced level of PGEM. Co-administration of esculetin (10 mg/kg—i.p.) and J77 decreased chemiluminescence. In AIA, J77 reduced mechanical hyperantinociception, edema, percentage of neutrophils, levels of MCP-1 and chemiluminescence. Co-administration of indomethacin and J77 reduced PGEM, MCP-1, and induced chemiluminescence levels. Celecoxib administered with J77 decreased PGEM and MCP-1 levels. Esculetin completely abolished the J77 antinociceptive effect produced. The antinociceptive effect of J77 in rat models of inflammatory nociception is in part secondary to its anti-inflammatory action but both periphery and central mechanisms are involved. Histamine receptor H_4 ligands interact with common NSAID and so may influence therapy and so this should be examined in more detail. *This project was financed by a grant from the National Science Center based on decision No DEC-2011/03/N/NZ4/03765.*

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CHARACTERIZATION OF HISTAMINE H_1 RECEPTOR EXPRESSION IN RAT EMBRYO CORTICAL AND MESENCEPHALIC NEUROEPITHELIA: IN THE SEARCH FOR ITS FUNCTIONAL ROLE DURING FETAL NEUROGENESIS *IN VIVO*

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The histamine H_1 receptor (H_1R) is expressed in the rat neural tube from embryo day 14 (E14) to E20. Histamine reaches its highest level during the neurogenic peak (E14) and has been shown to participate in neuronal differentiation through H_1R activation in vivo and in vitro, affecting in a contrasting manner cortical and ventral mesencephalic neuroepithelia neural stem cells. In the cerebral cortex, histamine increases FOXP2 neuron phenotype, a cortical deep layer phenotype implicated in speech in humans, ultrasonic sound generation in rodents and movement coordination. In ventral mesencephalic neural stem cells histamine promotes neuronal differentiation, but at high concentrations reduces the tyrosine hydroxylase phenotype in vivo and in vitro. Because histamine has been proposed to participate in neuronal commitment and the main cell population at E12 is neural stem cells, it is important to determine where in the cortical and ventral mesencephalic neuroepithelia the expression of H_1Rs, their cellular localization and the cell population that expresses the receptor. IR rat embryos of 12- and 14-day old were used to study the H_1R expression by RT-PCR, its membranal localization by binding assays. Additionally evidence of the presence of its protein in neural stem cells using double immunohistochemistry was studied to detect the H_1R and nestin which is a marker of neural stem cells. Our results showed H_1R expression in both neuroepithelia at E12 and E14, its presence in total forebrain/midbrain membranes with a higher density at E12 and co-localization with nestin. In conclusion, the H_1R is expressed by neural stem cells in the cortical and ventral mesencephalic neuroepithelia with a likely membranal localization, suggesting a functional role in cortical and mesencephalic development, a hypothesis that we have begun to study by blocking the effect of endogenous histamine at H_1Rs in 12 day-old rat embryos.

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DOPAMINERGIC REGULATION OF HISTAMINE NEURONS IN ZEBRAFISH

P. Panula, Y.-C. Chen

The brain histaminergic system is altered in diseases which affect the dopamine system. For example, levels of histamine and the density of histaminergic nerve fibers are increased in brains of Parkinson's disease patients. Translation inhibition of the second tyrosine hydroxylase (*th2*) in zebrafish leads to a decrease in dopamine content and an increased number of histamine neurons in developing zebrafish. We now sought to identify the mechanisms involved in this regulation. Treatment of the developing fish from 1 to 5 days post fertilization with 10 mM L-DOPA (precursor of dopamine), 10 uM SKF38393 (drd1-like receptor agonist) or 7.5 uM quinpirole (drd2-like receptor agonist) led to significant declines in the number of histamine neurons. We then analyzed which of the dopamine receptors (drd) are located in the posterior hypothalamus, where the histamine neurons are found. Dopamine receptors were cloned and the developmental expression patterns analyzed. *Drd1* was heavily expressed in hindbrain domains and retina, but not in the posterior hypothalamus. *Drd2a* was found throughout the brain, but not particularly strongly in the hypothalamus. *Drd2b* was found in the forebrain and hypothalamus, but not prominently in the caudal part. *Drd2l* was expressed heavily in

the diencephalon, most prominently in the hypothalamus and in the cell cluster which contains the histaminergic neurons. *Drd3* was expressed significantly in the forebrain and also in the hindbrain, whereas expression in the hypothalamus was modest. *Drd4* was expressed most prominently in the forebrain. The receptor expression patterns and drug treatment results support the concept that dopamine regulates the histaminergic neurons, most likely directly through dopamine receptors expressed in histaminergic neurons. Double in situ hybridization experiments and studies using dopamine receptor knockout fish are needed to verify the results. Multiple receptors could be involved, but the drd2l is the most likely candidate.

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LACK OF ANTIDEPRESSANT-LIKE EFFECT OF OLEOYLETHANOLAMIDE IN HISTAMINE-DEFICIENT MICE PARALLELS REDUCED HIPPOCAMPAL AND CORTICAL CREB PHOSPHORYLATION

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The failure of patients to respond to antidepressant drug therapy reveals the need to investigate alternative strategies and treatments for depression. Preclinical studies demonstrated that the endogenous agonist of Peroxisome Proliferator Activated Receptor-alpha (PPAR-α), Oleoylethanolamide (OEA) exerts an antidepressant-like effect. We previously have observed that neuronal histamine (HA) participates to OEA-induced central effects. Thus, we investigated if brain HA is also required for OEA-induced antidepressant-like effect in mice using the Tail Suspension Test (TST), a classical behavioural paradigm of antidepressant-like activity. Thus, mice unable to synthesize HA due to disruption of the histidine decarboxylase gene (HDC-KO) or to 5 μg i.c.v. injection of alpha-fluoromethylhistidine, a suicide inhibitor of this enzyme, were treated with OEA or vehicle. Results were compared to wild type (WT), and saline i.c.v. injected controls. In the TST, OEA (5 or 10 mg/kg i.p.; n = 5–12) significantly reduced immobility time in WT and control mice ($P < 0.001$); this effect was not observed in HA deficient mice. Imipramine (10 mg/kg i.p; n = 5–10) reduced immobility time of HA-deprived mice as well ($P < 0.001$). Neurochemical responses were studied by evaluating the phosphorylation level of cyclic AMP-response element binding protein (pCREB), a major player in the molecular mechanisms of antidepressant treatments. In normal mice but not in HA-deficient mice, OEA (10 mg/kg i.p.; n = 5 per experimental group) increased significantly cortical ($p < 0.05$) and hippocampal ($p < 0.01$) pCREB. As observed in behavioural responses, imipramine (10 mg/kg i.p.; n = 5) increased CREB phosphorylation independently of the presence of HA ($p < 0.05$). Hence, the neurochemical results parallel the behavioural data. We therefore suggest that OEA requires the integrity of the brain HA system to exert its antidepressant-like effects.

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THE ROLE OF HISTAMINE RECEPTORS IN THE CONSOLIDATION OF OBJECT RECOGNITION MEMORY

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Findings have shown that histaminergic system is involved on the consolidation, reconsolidation and extinction of different memories. Here we investigated in the CA1 region of the hippocampus the role of the histaminergic system on the consolidation of object recognition (OR) memory. For this, male *Wistar* rats (3 months; 300–330 g) with infusion cannulae stereotaxically implanted in the CA1 region of the dorsal hippocampus were trained in an OR task in the presence of two different objects (A and B). Twenty-four hours later, the animals were submitted to a test phase in the presence of a familiar and a new object (A and C). The training and test phase each lasted 5 min, during which the time that the animals spent exploring (sniffing and touching) the objects was recorded. The compounds to be tested were bilaterally infused into the CA1 region (1 μl/side) immediately, 30, 120 or 360 min after training. The infusion of vehicle or the H_1-receptor agonist, pyridylethylamine (10 mM), the H_2-receptor agonist, dimaprit (10 mM) and the H_3-receptor antagonist, thioperamide (50 mM), immediately, 30, 120, or 360 min did not affect the consolidation of the OR memory. However, the infusion of the H_1-receptor antagonist, pyrilamine (50 mM), the H_2-receptor antagonist, ranitidine (50 mM) and H_3-receptor agonist, imetit (10 mM), at 30 and 120 min after training impaired the consolidation of OR memory but not immediately or at 360 min. A similar result was observed after the infusion of the H_1-receptor antagonist, the H_2-receptor antagonist and the H_3-receptor agonist immediately and at 360 min after training. Therefore we have shown that OR memory was impaired by posttraining blockade of H_1 and H_2-receptors or by the

activation of H_3-receptors 30 and 120 min posttraining, that is, in a period when hippocampal memory consolidation takes place. This suggests that the histaminergic system participates in the consolidation of object recognition memory through the H_1, H_2, and H_3 receptors.

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MODULATION OF THE CONSOLIDATION OF SOCIAL RECOGNITION MEMORY IN TWO DISTINCT BRAIN AREAS

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Social recognition memory (SRM) is the ability to discriminate between familiar and unfamiliar conspecifics and is essential for social interaction, adaptive social behaviour, reproduction and survival. Here we investigate the involvement of H_2 histamine, β-adrenergic and D_1/D_5 dopamine receptors in the CA1 region of the hippocampus and in the basolateral amygdala (BLA) on the consolidation of SRM. For this, adult male *Wistar* rats (3 months old) with infusion cannulae implanted in the CA1 or in the BLA were subjected to a social discrimination task. The adults were habituated (20 min/day, for 4 consecutive days) to the experimental apparatus (open field arena) and 24 h after the last habituation session were exposed to a juvenile (male *Wistar* rats; 21 days old) for 1 h (sample phase). Twenty-four hours later, adults were subjected to a 5-min retention test in the presence of the previously presented juvenile (familiar) and a second juvenile (novel). The microinjections occurred immediately after the sample phase. Adults that received infusion of the vehicle (saline 0.9 %), the H_2 histamine receptor agonist dimaprit (Dima, 2.34 μg/side), the β-adrenoceptor antagonist timolol (Tim, 1.0 μg/side) or the D1/D5 dopamine receptors agonist SKF38393 (SKF, 12.5 μg/side) intra-CA1 or Dima, the β-adrenoceptor agonist isoproterenol (Iso, 10.0 μg/side) or the D1/D5 dopamine receptors antagonist SCH23390 (SCH, 1.50 μg/side) intra-BLA were able to recognize de familiar juvenile during the retention test. While adults that received infusion of the H_2 histamine receptor antagonist ranitidine (Rani, 17.54 μg/side), Iso or SCH intra-CA1 or Rani, Tim or SKF intra-BLA were not able to recognize the familiar juvenile during the retention test. This impairment to recognize the familiar juvenile was abolished by the co-infusion of Tim plus Iso, Rani plus Dima or SCH plus SKF into the CA1 or into the BLA. These results suggest that SRM is modulated by various systems in different ways.

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HISTAMINE H_3R EXPRESSION CHANGES IN AMYLOIDOPATHY AND MOUSE MODELS OF DEMENTIA

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Recent studies in Alzheimer's Disease mouse models and human cases, using ligand autoradiographical approaches, suggest that H_3R expression is largely unaltered or raised in some brain structures with age and disease progression. In this present study, we utilised our pan cross-species anti-H_3R and anti-r$H_{3A/C}$ antibodies both validated for selectivity by H_3R KO mice (C57BL background strain) using various immunobiochemical techniques. We investigated the time-course (3, 7, 12, 19 months) of H_3R expression in two distinct models of dementia, CD-1 mice and TASTPM APP/PS double mutant mice. These antibodies labelled three major H_3R isoform monomeric protein species (M_r 40,000, 43,000, 45,000, respectively) ($n = 4$ samples). Two-way ANOVA with post hoc test was used to assess statistical significance ($p < 0.05$ was deemed significant) Based on quantitative immunoblotting, using beta-actin as standard, we confirmed H_3R expression was largely unaltered in CD-1 mice in a range of brain structures between the ages 3 and 12 months; previously shown by our group, the latter age-point is where CD-1 mice display significant memory performance deficits. However, an apparent upregulation of H_3Rs appears to occur at the interim 7 month age point in both the cerebral cortex and hippocampus. This expression profile was not capitulated by the TASTPM mice where no significant differences were seen between 3 and 7 months of age, but a clear reduction was seen between 7 and 12 months of age; there is already significant amyloid load and memory deficits in the TASTPM model at the 7 month age point, which progressively worsens thereafter ($n = 3$–4 replicate animals). The maintenance or increase in H_3R expression seen may be the result of different compensatory mechanisms occurring in these two models; CD-1 mice do not display any detectable amyloidopathy up to at least 12 months of age, but has clear memory deficits at this age. Between 12 and 19 months of age, there appears to be a profound decrease in H_3R expression in both CD-1 and TASTPM mice. Both are likely to display, although not proven, profound memory deficits over this later age range. The Histamine H_3R is preserved or raised in the early stages of dementia providing a target for therapeutic intervention.

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METHYLPHENIDATE FACILITATES EXTINCTION LEARNING THROUGH β-NORADRENERGIC AND D1/D5-DOPAMINE RECEPTORS

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Methylphenidate (MPH), a dopamine and norepinephrine transporters inhibitor is the first-choice medication for attention-deficit/hyperactivity disorder. However, the mechanism(s) of action by which acute administration of MPH affects learning and memory is poorly understood. Here we investigated the effect of MPH in the CA1 region of the hippocampus on extinction learning. Male *Wistar* rats (3 months old; 330 g) with infusion cannulae stereotaxically implanted in the CA1 region were trained in a contextual fear conditioning (CFC) task, using 3 electrical foot shocks (0.5 mA, 2 s). Twenty-four hours later, animals were submitted to a 10-min extinction training (Ext Tr) of CFC, with the absence of the foot shock. After another 24 h, animals were placed again in the same apparatus for a 3-min extinction retention test (Ext Test). Animals that received intra-CA1 infusion of MPH (12.5 μg/side) 20 min before the Ext Tr expressed less freezing behavior than Veh-treated animals during both Ext Tr and Ext Test. Additionally, we investigated the involvement of β-adrenergic and D1/D5 dopaminergic receptors in this facilitatory effect on extinction learning induced by MPH. The administration of MPH + Timolol (1 μg/side; β-adrenergic antagonist) or MPH + SCH23390 (1.5 μg/side; D1/D5 dopaminergic antagonist) intra-CA1 20 min before the Ext Tr blocked the enhancing effect of the MPH on extinction learning. Whereas Timolol and SCH23390 infused intra-CA1 before the Ext Tr induced a decrease in freezing behavior in the Ext Tr and Ext Test compared to Veh group. These results suggest that MPH in the CA1 region of the hippocampus is able to facilitate extinction learning and this facilitatory effect occurs through both β-adrenergic and D1/D5 dopaminergic receptors.

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THE RELATIONSHIP BETWEEN PROTEIN SYNTHESIS AND PROTEIN DEGRADATION IN OBJECT RECOGNITION MEMORY

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For decades there has been a consensus that de novo protein synthesis is necessary for long-term memory. A second round of protein synthesis has been described for both extinction and reconsolidation. Recently, it was demonstrated that consolidation and reconsolidation depend not only on protein synthesis but also on protein degradation by the ubiquitin–proteasome system (UPS), a major mechanism responsible for protein turnover. However, the involvement of UPS on consolidation and reconsolidation of object recognition (OR) memory remains unclear. Here we investigated, in the CA1 region of the dorsal hippocampus, the involvement of UPS-mediated protein degradation in consolidation and reconsolidation of OR memory. For this purpose, male *Wistar* rats (3 months; 330 g) with infusion cannulae stereotaxically implanted in the CA1 region of the dorsal hippocampus were exposed to two different stimulus objects (A and B; sample phase). Twenty-four hours later, the animals were submitted to a reactivation phase in the presence of a familiar and a new object (A and C). Twenty-four hours later, they were subjected to a retention test with different combinations of objects (A and D; C and D; B and D). The sample, reactivation and test phase lasted 5 min each, during which was recorded the time that the animals spent exploring the objects. The microinjections into CA1 were carried out immediately after the reactivation phase. The UPS inhibitor β-Lactacystin (200 nmol/side) did not affect the consolidation and the reconsolidation of OR memory, while the protein synthesis inhibitor anisomycin (375 nmol/side) impaired the consolidation and the reconsolidation of the OR memory. However, β-Lactacystin was able to reverse the impairment caused by anisomycin on the reconsolidation process in the CA1 region of the hippocampus. Therefore, it is possible to postulate a direct link between protein degradation and protein synthesis during the reconsolidation of the object recognition memory.

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THE IMPAIRMENT OF AVERSIVE MEMORY OF RATS SUBMITTED TO NEONATAL MATERNAL DEPRIVATION WAS REVERTED BY HISTAMINE ACTING ON THE BASOLATERAL AMYGDALA

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Recent findings suggest a role of brain histamine in the regulation of memory consolidation, particularly in one-trial inhibitory avoidance (IA) learning and that disruption in the mother infant relationship i.e. maternal deprivation induces cognitive deficits. We investigated whether histamine itself, and histaminergic compounds given into the basolateral amygdala (BLA) immediately post-training can affect retention (24 h after training) of one-trial (IA) in rats submitted to early postnatal maternal deprivation. In all cases, deprived (Dep) animals (n = 9–12) had lower retention scores than non-deprived (N-dep) here considered controls group (n = 10–13). Histamine (10 nmol/0.5 µl/side) induced memory enhancement on its own in N-dep animals ($p < 0.01$) and was able to overcome the deleterious effect of Dep ($p < 0001$). The effect of SKF-91488 (50 nmol/0.5 µl/side) is similar to histamine ($p < 0.0001$) for both N-Dep and DEP rats. The H_3 agonist, imetit (10 nmol/0.5 µl/side) mimicked the enhancing effects of histamine ($p < 0.0001$); neither agonist H_1 pyridylethylamine (50 nmol/0.5 µl/side) nor the H_2 dimaprit (10 nmol/0.5 µl/side) had any effect. Ranitidine and thioperamide (50 nmol/0.5 µl/side) co-infused with histamine (10 nmol) fully blocked the restorative effect of histamine on retention in Dep animals ($p < 0.01$). Thioperamide, in addition, blocked the enhancing effect of histamine on memory of the N-dep animals ($p < 0.001$). None of the substances used when given into BLA had any effect on open-field or elevated plus-maze behavior in N-dep or Dep rats. Our results were represent median (±interquartile range) of step-down latencies during a memory retention test carried out 24 h after training, (n = number of animals per group). ***$p < 0.001$ and *$p < 0.5$, Saline vs. all treatment (one-way analysis of variance−ANOVA) in a non-parametric Kruskall–Wallis test followed by Dunn's multiple comparison test. These results suggest that the memory deficit induced by early postnatal maternal deprivation in rats may at least in part be due to an impairment of histamine H_3 receptor-mediated mediated mechanisms in the BLA. Our results are limited to experimental design in rats. These results shown the effective action of histamine inducing cognitive improvement or recover of fear memory. The extrapolation to humans requires further experiments, but is an interesting time-window to understand the receptor binding action and a possibly applicability in humans for treatment of posttraumatic stress disorder.

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HISTAMINERGIC MODULATION OF NOVELTY-INDUCED EXPLORATORY ACTIVITY

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Histamine (HA) is a neurotransmitter released in many brain regions including cortex, hippocampus, septum and amygdala. Along with the other aminergic nuclei, tuberomamillary histaminergic neurons regulate a variety of functions such as arousal and vigilance, homeostatic and cognitive functions. In the current study we analyzed horizontal and vertical locomotor activity of naïve male and female histidine decarboxylase knock out (HDC KO) mice in a novel environment. Animals were tested for 1 h for 3 consecutive days. HDC-deficient mice showed decreased exploratory rearings while female HDC KO mice also moved less than control mice. In contrast to previous studies the level of anxiety was not increased in KO mice. The habituation to the novel environment was also unaltered in HDC KO animals. In the next experiment we injected saline or amphetamine (AMPH, 5 mg/kg, salt) i.p. to HDC WT and KO male mice then exposed them to novel environment, tracked with Ethovision and videotaped behavior for 30 min. Locomotor activity (LA) was analyzed with Ethovision software and behavior quantified manually from video recordings. Trunk blood and brains were collected after the test. LA and behavioral pattern were similar in saline treated HDC WT and KO mice. Blood corticosterone level measured by HPLC was also unaltered in HDC KO mice. Injection of AMPH 5 mg/kg switched novelty-induced exploratory activity to persistent locomotion in control mice, while it induced stereotypy in HDC KO mice. An increasing body of evidence suggests an important role of HA in regulation of dopamine neurotransmission and thus LA, motivation and reward. One possible mechanism of histaminergic modulation of novelty-induced behavioral response is modulation of dopamine release via striatal histamine 3 receptors. Another possible way of exploratory behavior modulation is via interaction with other aminergic nuclei, e.g. the basal forebrain acetylcholine system.

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EXTINCTION LEARNING, WHICH CONSISTS OF THE INHIBITION OF RETRIEVAL, CAN BE LEARNED WITHOUT RETRIEVAL

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In the present study, we tested the hypothesis that extinction is not a consequence of retrieval in unreinforced conditioned stimulus (CS) presentation but the mere perception of the CS in the absence of a conditioned response. To test this hypothesis, male *Wistar* rats (3 months old, 330 g) with bilateral infusion cannulae implanted in the CA1 region of the hippocampus were trained in a contextual fear conditioning (CFC) task, using two electrical foot shocks (0.5 mA, 2 s). After 24 h, animals were subjected to a 20-min extinction training session (Ext Tr) of CFC, in the same conditioning chamber, without foot shocks. Twenty-four hours later, animals were subjected to a 3-min extinction retention test (Ext Test). In all sessions, the percentage of the time that animals spent freezing (no visible movement except for respiration) were measured. Animals that received intra-CA1 infusion of muscimol (Mus; 0.01 μg/side; agonist at γ-aminobutyric acid type A [$GABA_A$] receptors) 10 min before the Ext Tr expressed less freezing behavior than the Veh-treated (saline 0.9 %) animals during the Ext Tr. However, both groups exhibited similar levels of freezing during the Ext Test. Additionally, this inhibition of retrieval does not affect early persistence of extinction when tested 7 days later or its spontaneous recovery after 2 weeks. Furthermore, animals treated with Veh or Mus 10 min before the Ext Tr plus anisomycin (Ani; 80 μg/side; inhibitor of protein synthesis) or rapamycin (Rapa; 5 pg/side; inhibitor of mTOR [mammalian target of rapamycin]) immediately after the Ext Tr, expressed higher levels of freezing behavior than the Veh group during the Ext Test, indicating that even in the absence of retrieval, Ani and Rapa were able to impair the consolidation of the extinction of CFC. These findings indicate that behavioral expression during an unreinforced retrieval session is not necessary for the initiation, maintenance or spontaneous recovery of the extinction learning.
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PACAP MODULATES THE CONSOLIDATION OF SOCIAL RECOGNITION MEMORY

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic bioactive peptide that has a broad spectrum of biological functions including neurotransmitter, neurotrophic and neuroprotective. Moreover, it has been suggested that PACAP plays an important role in the modulation of social behavior as well as in the consolidation and extinction of fear conditioning memory. In the present study, we investigated the involvement of PACAP in the CA1 region of the dorsal hippocampus and in the basolateral amygdala (BLA) on the consolidation of social recognition memory. For this, adult male *Wistar* rats (3 months old) with bilateral infusion cannulae implanted in the CA1 or in the BLA were subjected to a social discrimination task. Adult animals were habituated (20 min per day, for 4 consecutive days) to the experimental apparatus (open field arena) and 24 h after the last habituation session were exposed to a juvenile (male *Wistar* rats; 21 days old) for 1 h (sample phase). Twenty-four hours later, adults were subjected to a 5-min retention test in the presence of the previously presented juvenile (familiar) and a second juvenile (novel). Adults that received infusion of PACAP 6–38 (40 pg/side; antagonist of PACAP) intra-CA1 or intra-BLA immediately after the sample phase were not able to recognize the familiar juvenile during the retention test, as well as the adults that received PACAP 6–38 (40 pg/side) intra-BLA 60 min after the sample phase. Interestingly, this impairment to recognize the familiar juvenile was abolished by the co-infusion of PACAP 6-38 plus *S*-Nitroso-*N*-acetyl-DL-penicillamine (SNAP; 5 μg/side; nitrous oxide donor). These results suggested that PACAP participates in the consolidation of the social recognition memory, and that this effect seems to occur through the action of nitric oxide.
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ELECTROENCEPHALOGRAM OF H_1KO MICE UNDER ISOFLURANE ANAESTHESIA

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The inhaled anaesthetic isoflurane may exert loss of consciousness (LOC) via the modulation of sleep/arousal neural pathways. One of the pathways which is important for evoking and maintaining arousal is the histaminergic neural system. We found that the histamine H_1 receptor was involved in LOC induced by isoflurane from the results H_1KO mice behavioural experiments. However, there was no evidence that H_1 receptors in the cerebrum were actually involved in LOC, because animal behaviours may be a reflection of musculoskeletal abnormalities. The aim was to elucidate whether cerebral H_1 receptors are involved in LOC induced by isoflurane induced general anaesthesia. We measured in vivo electroencephalogram (EEG) under various concentrations of isoflurane. H_1KO and WT mice ($n = 4$) underwent EEG surgery according to the manufacturer's protocol (Pinnacle Technology, KS, USA). Basically the mice were anaesthetized with pentobarbital and mounted in a stereotaxic device (Kopf, CA, USA). A mouse EEG head-mount (Pinnacle technology) was implanted onto the skull with four intracortical screws. EEG screws were positioned in the frontal and parietal cortices. 2 weeks later, EEGs were sampled for 5.5 h using Sirenia software (Pinnacle technology). Data were analyzed by SleepSign (Kissei Comtec, Nagano, Japan) with manual adjustments. We found that the EEG from H_1KO mice changed more rapidly to slow wave (such as theta and delta) when compared to WT during the induction phase of general anaesthesia. In the maintenance phase, H_1KO mice showed planarized EEG recording whereas the WT had burst-suppression and/or spike-and-wave pattern, indicating H_1KO brain activity was strongly suppressed by isoflurane. The recovery from flat/slow wave pattern after withdrawal of isoflurane was prolonged in H_1KO group. The EEG analysis indicated that isoflurane suppressed neuronal activity in H_1KO more strongly.

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Histamine H_3 receptor

HISTAMINE H_3 RECEPTOR ANTAGONISTS—FROM BENCH TO BEDSIDE AND BACK TO BENCH

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Histamine H_3 receptor ligands have been under investigation for several decades. Initially it started with the derivatization of the endogenous ligand histamine. With imidazole-containing ligands as lead structure, numerous selective and potent ligands have been developed (e.g. thioperamide, clobenpropit, ciproxyfan). The development of non-imidazole lead structures as antagonists/inverse agonists gave fresh impetus to drug optimization. Several related parent structures have been studied for pharmacokinetics and later also pharmacodynamics and toxicological optimization. Pitolisant (Wakix®) has been the first important milestone making it into market for narcolepsy as an orphan drug. The different sleep reducing, procognitive and other effects in the central nervous system opened the potential for the therapeutic treatment of a number of different central disorders. Although this potential is clear, it is unclear if the effects are robust enough for overcoming the different therapeutic gold standards. In that direction, others and we and others have developed several new classes of histamine H_3 receptor antagonists with additional properties leading to multitargeting compounds. The polypharmacological approach offers the chance for highly disease oriented therapeutic drug treatment.

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MOLECULAR PHARMACOLOGY OF THREE ZEBRAFISH H_3-LIKE RECEPTORS

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Zebrafish (*Danio rerio*) has emerged as an animal model for neurodegenerative diseases due to the resemblance of its brain structures and aminergic systems with those of mammals. Three histamine receptors (H_1R, H_2R and H_3R) have been described with a sequence similarity of around 40–50 % with their human orthologs. Previously, we reported on the cloning of two additional zebrafish H_3-like receptors and showed that all three zfH_3R subtypes were able to bind the agonist *N*-α-methylhistamine ([^{3}H]-NαMH) with similar K_D values (~2 to 3 nM). Competition binding experiments with 20 human H_1R, H_2R, H_3R, and H_4R ligands revealed that zfH_3Rs show distinct profiles. However, no information about the functional profiles of these ligands and the signalling of the zfH_3Rs was known. In this study we investigated zfH_3Rs-mediated signalling pathways and characterize several ligand profiles. Real-time cAMP measurements using the BRET-based biosensor CAMYEL showed that zfH_3Rs coupled to $G\alpha_{i/o}$ proteins and agonists and antagonist were identified for each of the three receptors. This study opens the opportunity to discover novel compounds that can discriminate between the zfH_3Rs subtypes to explore their roles in zebrafish physiology.

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ON THE EXISTENCE OF A HISTAMINE H_3-ADENOSINE A_{2A} RECEPTOR HETEROMER

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The striatum plays a major role in the modulation of motor and cognitive processes and its deregulation leads to pathologies such as Parkinson's disease and addictive behaviors. The $G\alpha_{i/o}$-coupled histamine H_3 receptors (H_3Rs) are ubiquitously expressed in the striatum and inhibit GABAergic transmission in this nucleus. The striato-pallidal neurons distinctively express $G\alpha_s$-coupled adenosine A_{2A} receptors ($A_{2A}Rs$) which inhibit the intrastriatal but paradoxically facilitate the striato-pallidal GABA release. H_3Rs and $A_{2A}Rs$ can form dimers with dopamine D_2 receptors, these interactions being potential targets for the treatment of addiction and Parkinson's disease, respectively. We hypothesize that H_3Rs also interact with $A_{2A}Rs$ and that these heteromers will display a unique pharmacology. We used a multi-pronged approach to determine the $A_{2A}R$-H_3R physical interaction and functionality in HEK-293 cells and in rat striatal synaptosomes. In the cells, we first found H_3R-$A_{2A}R$ dimerization with a chimeric G-protein complementation assay. Using a Glosensor cAMP assay we found that H_3R activation diminished the potency of the $A_{2A}R$ (pEC_{50} control 7.80 ± 0.30, +RAMH 6.90 ± 0.34) and increased the E_{max} value from 195 ± 9 % to 314 ± 13 % of basal (n = 3, $P = 0.0007$). $A_{2A}R$ activation completely prevented H_3R-mediated cAMP inhibition (Imax 63 ± 4 %, pIC_{50} 8.58 ± 0.36). By using striatal synaptosomes we confirmed the physical presence of H_3R-$A_{2A}R$ heteromers by coimmunoprecipitating H_3Rs with $A_{2A}Rs$. In binding assays H_3R activation decreased the affinity of synaptosomal

A_{2A}Rs (pKi 8.10 ± 0.04, +RAMH 7.7 ± 0.04) and in cAMP assays prevented A_{2A}R-induced cAMP formation. The activation of A_{2A}Rs or H_3Rs inhibited GABA uptake by striatal synaptosomes to a similar extent (8 ± 2 % and 12 ± 3 % of control values, respectively), but co-activation resulted in an increased inhibition (22 ± 3 %, n = 3, $P = 0.0038$). These data support the existence of a striatal A_{2A}R-H_3R heterodimer capable of modulating the intrastriatal transmission.

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A BRET-BASED KINETIC LIGAND-RECEPTOR BINDING ASSAY FOR THE HISTAMINE H_3 RECEPTOR

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Nowadays, ligand-receptor binding kinetics and drug-target residence time are widely accepted as important parameters for lead compound optimization in addition to the classically used binding affinity. Traditionally, radiolabeled ligands are used to determine association and dissociation rate constants of ligands either directly or indirectly via a competition association assay. Recently, a novel bioluminescence resonance energy transfer (BRET) method was introduced that measures fluorescent ligand binding to a NanoLuc-fused receptor. With this BRET method, real-time binding on living cells can be measured, and therefore results might better reflect in vivo conditions as compared to the routinely used cell homogenates or purified membrane fractions in high-throughput binding assays. Since ligand-receptor kinetics has established its importance in drug discovery, we aimed to setup a BRET-based kinetics assay using a NanoLuc-fused histamine H_3 receptor. In this study, we were able to measure association and dissociation rate constants of fluorescent labeled ligands and determine the kinetic parameters of several unlabeled ligands via a competition association assay in real-time on NanoLuc-fused histamine H_3R, allowing us to compare ligand-receptor residence time for a set of histamine H_3 receptor ligands.

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PHARMACODYNAMICS (PD) AND PHARMACOKINETICS (PK) OF SINGLE DOSES OF THE NOVEL H_3-RECEPTOR ANTAGONIST IRDABIS (CEP-26401) IN COMPARISON WITH MODAFINIL AND DONEPEZIL IN HEALTHY VOLUNTEERS (HVs)

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H_3R-antagonists are in development for various disorders with impaired cognition or hypersomnia, but clinical results are mixed. Previous studies with the H_3R-antagonist irdabisant showed slight cognitive improvement in HVs, but only at the lowest 20 μg dose. Higher doses caused sleep deficits, but no cognitive effects. Detailed CNS profiles of low doses of irdabisant vs modafinil and donepezil were compared. In this double-blind, placebo/positive-controlled, partial 6-way crossover study, 40 HVs were randomized to placebo, irdabisant (5, 25 or 125 μg), modafinil 200 mg or donepezil 10 mg in the morning, followed by frequent PD and PK, and polysomnography overnight. PD was assessed with the NeuroCart, a drug-responsive battery of neurophysiological (EEG, saccadic (SPV) and smooth pursuit eye movements), cognitive (verbal learning and *n*-back), performance (adaptive tracking, body sway, reaction time (RT)) and subjective tests (VAS for aspects of mood, calmness, alertness, task enjoyment and psychotic-like). CANTAB was used for stop-signal inhibition, rapid visual information processing, paired associate learning (PAL) and spatial working memory. Almost every irdabisant dose caused significant changes in most NeuroCart tests. Subjective effects, reflecting increased energy, alertness, mood and task enjoyment, were largest at 25 μg. Objective improvements of tracking, sway, SPV, RT during *n*-back and EEG γ power were dose-related. At the highest dose, memory worsened slightly. Sleep reduction was also dose-related. Modafinil had similar effects, but irdabisant's subjective 'energizing' profile had a more relaxed undertone. Donepezil did not improve any test. Irdabisant 25 μg had the best balance between CNS stimulation and sleep suppression. The results suggest that very low irdabisant doses cause an optimal CNS stimulation, while higher dosages may cause overstimulation and impaired sleep. Whether irdabisant can have beneficial clinical effects remains to be studied.

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THE HISTAMINE H_3-RECEPTOR PARTIAL AGONIST OXATHRIDINE SHOWS SEDATIVE EFFECTS BUT ALSO PAEUDO-HALLUNCINATIONS: FIRST-IN-HUMAN RANDOMIZED, PLACEBO CONTROLLED, SAD STUDY IN HEALTHY VOLUNTEERS

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Oxathridine (BP1.5375) is a selective partial agonist of the histamine H_3 receptor (H_3R). It is potentially useful for the treatment of brain disorders such as sleep disorders. Preclinical data indicate that oxathridine easily enters the brain and increases deep sleep. This first-in-human study was designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of oxathridine in healthy male subjects after single oral administrations, as compared to placebo. In this double-blind, placebo-controlled, randomized study (NCT01965301 in Clinicaltrials) 40 healthy male subjects in 5 cohorts received single doses of oxathridine or placebo (6:2). Oxathridine plasma concentrations and safety were determined. NeuroCart® neurocognitive tests were performed at regular intervals. Doses ranging from 0.25 to 5 mg oxathridine were investigated, behaving dose-linearly for C_{max} and AUC, with a half-life of 2.5 h. Significant effects compared to placebo were seen in the 2.5 and 5 mg group on adaptive tracking (−4.94 and −4.81 %), body sway (34.6 % and 43.0 %) and saccadic peak velocity (−37.4 and −22.5 deg/s). A significant effect compared to placebo in perception, a.o. VAS external (0.330 log mm), was seen only in the 5 mg group. Further dose escalation was prevented by side effects observed in the higher doses, including (orthostatic) hypotension (1.5, 2.5 and 5 mg) and pseudo-hallucinations (5 mg only). There were significant sedative effects at 2.5 and 5 mg for the first hours after dosing, but these effective doses were also accompanied with adverse effects e.g. orthostatic hypotension and pseudo-hallucinations. The hallucinatory activity elicited by H_3R stimulation together with the anti-hallucinatory activity of pitolisant in narcolepsy suggest a role of histaminergic systems in this cognitive parameter. Based on the NeuroCart® test battery, there was no clear separation between doses that caused sedation and adverse effects.

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HISTAMINE H_3 RECEPTOR ANTAGONISM IS ENHANCED BY PHARMACOLOGICAL ASSOCIATION WITH NO DONORS IN NEW ZEALAND WHITE RABBIT MODELS OF GLAUCOMA

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Glaucoma is the second leading cause of blindness worldwide, characterized by progressive optic nerve atrophy; it is caused by elevated intraocular pressure (IOP). IOP reduction is the only therapeutic approach demonstrated to preserve visual function in patients affected by glaucoma. The first line treatment consists of topical IOP-lowering drugs, usually as monotherapy. A significant number of patients require more than one drug to reach a target IOP. We previously demonstrated that the topical treatment with H_3R antagonists is effective in reducing IOP in rabbit models of glaucoma. Prostaglandin analogues (PGAs) are the most effective IOP lowering agents available in the market. Nitric oxide donors are successfully associated with PGAs in the treatment of glaucoma.

Aim of this research was to evaluate the potential NO-donor boost effect on IOP reduction obtained by ciproxifan.

Ocular hypertension was obtained by the injection of 50 μl of hypertonic saline (5 %) into the vitreous or by 100 μl (0.1 %) of Carbomer in the anterior chamber. IOP measurements were performed using applanation tonometry (Tono-Pen AVIA®, Reichtert, USA) prior to saline or Carbomer injection (baseline), immediately before drug dosing (pre-treatment) and 1–4 h after saline injection in the acute model and after 24 h in chronic model. All the animals underwent Ecocolor Doppler evaluation before and after chronic drug treatment, Pourcelot Resistance Index (RI) was calculated.

IOP rose from 16.4 ± 3.2 mmHg at baseline to 39.4 ± 4.8 mmHg after hypertonic saline injection and from 14.2 ± 5.3 to 36.8 ± 5.6 four days after Carbomer injection. Ciproxifan and molsidomine (0.1, 0.3, 0.5, 1 %) dose-dependently reduced IOP at 60' after saline injection (0.5 % p value <0.05, 1 % p value <0.01). The ciproxifan/molsidomine combination that produced the higher IOP reduction was 0.5/0.5 %. IOP and mean resistance index (RI) of retinic artery were significantly reduced by this combination in the chronic model setting (p value <0.01 at day 10, 12 and 13 for IOP; p value <0.05 for RI).

Histaminergic H_3R antagonists confirmed to be an interesting new therapeutic option for the treatment of

glaucoma and the association with a NO-donor compound showed a synergic effect boosting the IOP lowering efficacy and ameliorating the vascular performance of the retinic artery.

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DIFFERENTIAL HOMOLOGOUS DESENSITIZATION OF THE HUMAN HISTAMINE H_3 RECEPTORS OF 445 AND 365 AMINO ACIDS

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Histamine H_3 receptors (H_3Rs) are found in neuronal cells as auto- and hetero-receptors. Alternative splicing of the human H_3R (hH_3R) could generate at least 20 isoforms, and in addition to the hH_3R_{445} an isoform of 365 amino acids (hH_3R_{365}) is widely expressed. Homologous desensitization, a major mechanism to regulate the function of G protein-coupled receptors (GPCRs), is triggered by the phosphorylation of activated receptors by GPCR kinases (GRKs) and we have shown that the hH_3R_{445} stably expressed in CHO-K1 cells experiences homologous desensitization. The hH_3R_{365} lacks 80 residues in the third intracellular loop, an important region for GPCR coupling to G proteins, as well as for the phosphorylation by GRKs in the homologous desensitization mechanism. In this work, we aimed to study whether the hH_3R_{365} experiences homologous desensitization and the possible differences with the hH_3R_{445} isoform. Both hH_3R isoforms were stably expressed in CHO-K1 cells, receptor levels were determined by [^{3}H]-*N*-α-methyl-histamine binding assays and functional desensitization was evaluated in cells pre-incubated with the H_3R agonist *R*-α-methyl-histamine (RAMH). The lack of 80 residues in the hH_3R_{365} had no effect on the expression by CHO-K1 cells, the affinity for selective ligands or the presence on the cell surface. Pre-incubation for 30 min with RAMH (1 μM) reduced by 58 ± 8 % hH_3R_{365} Gαi/o-mediated signaling and resulted in the loss of receptors from the cell surface, as well as in reduced affinity for immepip. Maximal functional desensitization differed in both the extent (96 ± 15 and 58 ± 8 % for hH_3R_{445} and hH_3R_{365}, respectively) and the length of exposure required (60 and 30 min). Furthermore, at 60 and 120 min of RAMH pre-incubation, the hH_3R_{365} showed partial re-sensitization, whereas the hH_3R_{445} remained desensitized. These results indicate that the hH_3R_{365} experiences homologous desensitization, but the process differs from the hH_3R_{445} in time-course, magnitude and re-sensitization.

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EVALUATION OF HISTIDINE DECARBOXYLASE ACTIVITY AND HUMAN HISTAMINE H_3 RECEPTOR (H_3R) AND HISTIDINE DECARBOXYLASE (HDC) mRNA LEVELS

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Histamine plays pleiotropic roles in human pathophysiology that are dependent of both the capability to be synthesized in a given environment and the cell-specific combination of histamine receptors expressed in the different human cell types. At present, characterization of the molecular bases of the tissue-specific roles of the histamine metabolism-related elements (HMRE) requires further research efforts in order to fully explain the multiple histamine roles in human pathophysiology and to develop new intervention possibilities derived from this knowledge. To this purpose, we have developed some methods for years to approach expression estimation of histamine HMRE in different human cell types and tissues. They are a radiolabelled method for mammalian histidine decarboxylase (HDC) activity measurement, and qRT/PCR methods for quantification and transcript analyses of human HDC and human histamine H_3 receptor (H_3R). As a result, we have provided an interesting and reproducible tool for the analysis of the HMRE to gain insight into histamine roles in human tissues and cell types.

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CHARACTERIZATION OF N-SUBSTITUTED-N-[4-(4-(7-PHENOXYHEPTYL) PIPERAZIN-1-YL)BUTYL]GUANIDINES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS

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Histamine H_3 receptors (H_3R) are involved in the central and peripheral regulation of histamine levels. By postsynaptical modulation they regulate the levels of other

neurotransmitters, such as ACh, 5-HT, NA and NPY. Therefore, the blockade of H_3R might provide useful pharmacological target in the treatment of CNS-based diseases, which has been confirmed by intensive pharmacological studies. In present study, we evaluated the group of *N*-substituted-*N*-[4-(4-(7-phenoxyheptyl)piperazin-1-yl)butyl]guanidine derivatives as a potent H_3R antagonists. Considered structures were selected basing on preliminary screening results obtained in electrically evoked contraction of the guinea-pig jejunum assay. The antagonistic properties at H_3R were confirmed in cAMP accumulation assay. Moreover, radioligand binding assays towards H_3R and off-target histamine H_4 receptor (H_4R) allowed for determination of respective K_i values and consequently enabled the characterization of compounds selectivity. For two selected compounds the following safety parameters were evaluated in vitro: hERG channel inhibition (automated patch-clamp technique), mutagenicity (Ames test) and hepatotoxicity (proliferation assay at human HepG2 cells).Evaluated guanidines showed affinity towards histamine H_3R in submicromolar concentration range and represented up to 20-fold selectivity over H_4R. Moreover, the considered structures showed good safety index in performed experiments and therefore could be considered as a promising structural scaffold for further development of H_3 histamine receptor ligands.

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NEW N-(4-PHENOXYALKYLPIPERAZIN-1-YL)ALKYLGUANIDINES, POTENT AND BRAIN-PENETRATING NON-IMIDAZOLE HISTAMINE H_3-ANTAGONISTS

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The guanidine derivatives, both imidazole and non-imidazole class, are potent and selective histamine H_3 receptor antagonists. Previously, we reported the synthesis and biological evaluation of *N*-substituted-*N*-[ω-(ω-phenoxy-heptylpiperazin-1-yl)butyl]guanidines. Detailed structure–activity studies revealed that a halogen at position 4 in the benzene ring leads to compounds with lower activity ($pA_2 = 7.89$–7.80) than their unsubstituted analogue ($pA_2 = 8.21$). Here we report on further in vivo pharmacological studies that were done for *N*-benzyl-*N*-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine and *N*-4-chlorobenzyl-*N*-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine. Lewis rats, males, 8-wk. old, were placed and kept individually in metabolic cages, their fluid and feed consumptions were recorded daily in the morning. After 3 days with no treatment (control period) they were randomly given one of the two compounds or Ciproxifan hydrogen maleate as a reference H_3R antagonist (3 mg/kg s.c., each). The treatment was continued for 5 days. The following morning, after consumptions recording, the rats were decapitated, their brains collected frozen and kept at −80 °C until binding studies were performed. Histamine H_3 receptors were assayed with $[^3H]$ *R*-α-methyl histamine ligand. The results obtained indicate that the examined compounds penetrate the blood–brain barrier, with their H_3R antagonistic activity being lower (*N*-benzyl-*N*-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine) or similar (*N*-4-chlorobenzyl–*N*-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine) to that of Ciproxifan. This work was supported by MUL (i.e. Med Uni Lodz) 503/5-087-02/503-01 and 500/3-016-01/503-31-001.

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DIFFERENCES IN β-ARRESTIN2 RECRUITMENT BY HUMAN H_3R ISOFORMS AS DETERMINED BY ENZYME FRAGMENT COMPLEMENTATION

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The human H_3 receptor (hH_3R) receptor gene encodes for 20 hH_3R isoforms as a consequence of alternative splicing and they are differentially expressed within the CNS. The hH_3R-445 -415, -365 and -329 isoforms conserve the prototypical 7TM domains, but have different IL3 loop truncations. Therefore, they are known to have different pharmacological and signaling profiles. Although hH_3R isoforms are known to signal via Gαi, it is unknown if they can mediate G protein-independent signaling and regulation (i.e. desensitization and internalization via β-arrestin1/2). For the closely related human H_4 receptor (hH_4R) (~60 % amino acid sequence similarity) β-arrestin2 recruitment was recently reported. In this study, β-arrestin2 recruitment at hH_3R isoforms is compared using Enzyme Fragment Complementation technology. Our results indicate differences in pharmacological profiles (i.e. potency, efficacy and mode of action) of hH_3R ligands between hH_3R isoforms. For instance, H_3 ligand immepip (full agonist in G protein signaling) was found to be a partial agonist for hH_3R-445 ($\alpha = 0.4$), hH_3R-415 ($\alpha = 0.5$), hH_3R-365 ($\alpha = 0.8$) and hH_3R-329 ($\alpha = 0.9$). Moreover, imetit (full agonist in G protein signaling) was found to be a selective agonist for hH_3R-365 and hH_3R-329, but an

antagonist for hH_3R-445 and hH_3R-415. Stimulation with agonists N-alpha-methylhistamine (NαMH) and R-alpha-methylhistamine (RαMH) resulted in higher potencies for hH_3R-365 and hH_3R-329, as compared to hH_3R-445 and hH_3R-415. This study reveals differences in pharmacological profiles of H_3 ligands on β-arrestin2 recruitment between hH_3R isoforms, presumably caused by differences in the IL3 loop.

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HISTAMINE H_3-RECEPTORS MAY BE INVOLVED IN THE PRODUCTION OF CYTOKINES AND CHEMOKINES DURING MUSIC THERAPY AND OTHER NON-DRUG INFLUENCES

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The discovery of histamine H_3-receptors as presynaptic autoreceptors on histamine-containing neurons and on peripheral tissues support an idea that histamine effects during non-immunological, non-drug influences respond partly via this type of receptor. Furthermore, diverse expression of H_3-receptors throughout the brain can modulate the release many neurotransmitters. Our previous investigations have demonstrated that antagonists of H_3-receptors modulate pro- and anti-inflammatory cytokine synthesis in patients with acute sensorineural hearing loss (ONT). It is well known that non-drug factors can produce equivalent outcomes of the clinical symptoms producing a much shorter onset time and less overall risk and cost, than pharmacological therapies. One of such non-medication factors is music therapy (MT). Effects of MT on behavioral and psychological symptoms have been demonstrated in various clinical situations. MT has been used before and after surgical interventions, in psychotherapy of behavior disturbances such as aggression, disruption, shadowing, depression, repetitive behaviors and in complex management of dementia. It has been shown that music enhances a variety of cognitive functions e.g. attention, learning, communication and memory. Recent studies have demonstrated that music can decrease cortisol and adrenocorticotropin releasing hormone (ACTH). In our previous studies we have demonstrated that different types of music may influence salivary histamine secretion in allergic and non-allergic young volunteers. The aim of this study was to demonstrate that MT and ONT influence H_3-receptor activity and regulation. Using peripheral blood mononuclear cells (PBMC) and dendritic cells derived from PBMC of 12 healthy volunteers cultivated with $H_{3/4}$ dual antagonist Ciproxifan (10^{-5} M) as well as $H_{3/4}$ agonist *N*-Methyl-1*H*-imidazole-4-ethanamine (Na-methylhistamine) (10^{-5} M) using multiplex technology, we found that histamine modulated the production of multiple immunoregulatory cytokines, chemokines and growth factors via H_3-receptors.

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Histamine H_4 receptor

THE HISTAMINE H_4 RECEPTOR MEDIATES CHEMOTAXIS OF HUMAN LUNG MAST CELLS

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Previous studies by others have shown that histamine induces chemotaxis of mouse mast cells by engaging the H_4 receptor (H_4R). The aim of the present study was to determine whether the H_4R mediates chemotaxis of human lung mast cells (HLMC). HLMC were generated by physical and enzymatic disruption of lung tissue obtained from surgical resections. Isolated HLMC were further purified by Percoll density gradient centrifugation and chemotaxis assays performed using ChemoTx® system (8 µm pore size). On average, 20,000 HLMC were loaded per condition, with or without the selective H_4R agonist JNJ28610244 (10 µM). Stem Cell Factor (SCF, 10 ng/ml), a known HLMC chemoattractant, was used as a positive control. The plate was incubated (4 h, 37 °C) in a CO_2 incubator, and the number of mast cells migrating to the bottom chamber evaluated using the mast cell specific stain, alcian blue. The effects of the selective H_4R antagonist JNJ7777120 (10 µM) against JNJ28610244 were also investigated. In additional functional studies, HLMC were incubated (25 min) with JNJ28610244 (10 µM) and histamine released into the supernatants assayed using an automated fluorometric technique. ANOVA was performed (GraphPad V6) to determine whether the effects were significant. JNJ28610244 was an effective inducer ($P < 0.001$) of HLMC chemotaxis, causing a 3-fold increase in migration over control ($n = 9$). SCF was at least as effective ($P < 0.001$) at inducing mast cell migration ($n = 17$). In further studies ($n = 4$), the H_4R antagonist JNJ7777120, reversed chemotaxis induced by JNJ28610244 by ~64 % ($P < 0.05$). In functional studies ($n = 11$) JNJ28610244 did not induce histamine release in HLMC. These data indicate that activation of the H_4R mediates chemotaxis of HLMC.

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HUMAN H_4R SIGNALING AND DESENSITIZATION

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The human histamine 4 receptor (hH_4R) is mainly expressed on cells of hematopoietic origin and involved in allergic and inflammatory diseases. Agonist-induced activation of G protein-coupled receptors (GPCRs) leads to signaling via G proteins and subsequent receptor desensitization by G protein-coupled receptor kinases (GRKs) and β-arrestin recruitment. In this study, signaling and desensitization of the hH_4R is investigated by making use of fusion constructs and bioluminescence resonance energy transfer (BRET)-based assays in response to chemically distinctive hH_4R agonists. This study shows that the hH_4R signals via $G\alpha_{i1}$, $G\alpha_{i2}$ and $G\alpha_{i3}$ proteins without preference towards a specific isoform. Signaling regulated by other G protein subtypes, respectively $G\alpha_s$, $G\alpha_{13}$ and $G\alpha_q$, could not be observed. Furthermore, activation of the hH_4R leads to rapid translocation of intracellular GRK2 and 3 towards the cell membrane and β-arrestin1 and 2 recruitment, demonstrating the occurrence of receptor desensitization. Understanding the molecular aspects of both the signaling and regulatory mechanisms of the hH_4R are important for drug development.

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THE PHARMAOCLOGY AND CLINICAL DEVELOPMENT OF TOREFORANT, A HISTAMINE H_4 RECEPTOR ANTAGONIST

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Toreforant (JNJ 38518168) is a potent antagonist of the H_4R with an inhibition constant (Ki) of 8.4 nM at the human H_4R and greater than 25-fold selectivity over other histamine receptors. The compound is less potent at the mouse H_4R with a Ki of approximately 300 nM. Toreforant is efficacious in mouse models of arthritis, asthma and dermatitis. In a mouse CIA model treatment of animals with toreforant at 100 mg/kg BID lead to a reduced manifestation of disease as judged by the severity score and blinded histological analysis of joint pathology. Repeat-dose chronic toxicity studies in rats (6 months of daily dosing) and monkeys (9 months of daily dosing) indicated an excellent safety profile supporting the clinical testing of the compound. An oral formulation of toreforant was studied in a phase 1 human volunteer study to assess safety, pharmacokinetics and pharmacodynamics. The compound was well-tolerated at all doses tested and exhibited good pharmacokinetics upon oral dosing. In addition, dose dependent inhibition of histamine-induced eosinophil shape change was detected suggesting that the H_4R was inhibited in vivo. Phase 2 clinical studies have been conducted in patients with rheumatoid arthritis, asthma or psoriasis.

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CHARACTERIZATION OF BEHAVIORAL PHENOTYPES IN HISTAMINE H_4-RECEPTOR KNOCKOUT MICE

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Histamine mediates a wide range of physiological functions through binding to four known histamine receptors. The recently cloned histamine receptor H_4 is functionally expressed in the central nervous system, although this remains controversial. The aim of this study was to characterize the behavioral phenotypes of H_4R knockout mice to understand the role of H_4R in mediating histamine effects. We characterized the behavior of H_4R-KO and wild-type mice by subjecting them to a range of behavioral tests ($n = 10$ per genotype, 3 months of age). H_4R-KO mice showed a slightly increased spontaneous mobility and exploratory activity in the hole board test, without any deficit in the rotarod test. The H_4R-KO mice had no cognitive impairment in the novel object recognition and passive avoidance tasks. However, KO mice differed from their WT in the mood-related behavioral tests. H_4R-KO mice exhibited an anxiety-like phenotype in the light/dark box test ($P < 0.01$) and, a trend toward a depressive-like phenotype in the tail suspension test ($P < 0.05$). We also examined whether food intake changes under a condition of food deprivation, were altered in H_4R-KO mice. H_4R-KO mice manifested orexigenic phenotype that is characterized by increase in food intake in non-food deprived mice ($P < 0.001$). H_4R-KO mice exhibited normal pain sensitivity after thermal and mechanical stimulus. We have previously demonstrated that neuronal H_4R is involved in neuropathic pain transmission. To validate the involvement of H_4R in pain sensation, Von-Frey and Plantar tests were performed before and after spared nerve injury (SNI) in H_4R-KO mice. We found that H_4R-KO mice that underwent SNI surgery showed decreased ipsilateral paw withdrawal threshold ($P < 0.01$), indicating the important role of H_4R in the histamine-mediated modulation of pain transmission. The behavioral characterization of H_4R-KO mice has demonstrated important roles of neuronal H_4R in the regulation of behaviors like anxiety, regulation of food intake and pain perception, suggesting that neuronal histamine is a mediator of these neuronal functions through H_4R in mice.

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THE SELECTIVE HISTAMINE H_4 RECEPTOR ANTAGONIST JNJ 777120 IS PROTECTIVE IN A RAT MODEL OF TRANSIENT CEREBRAL ISCHEMIA

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Histamine is an important neurotransmitter or neuromodulator in the Central Nervous System (CNS). Recently, numerous studies have suggested that histamine and its receptors play important roles in cerebral ischemia. In the experimental model of focal cerebral ischemia induced by occlusion of the middle cerebral artery (MCAo) in rats, the levels of histamine evaluated by microdialysis increase in the ischemic areas. The human histamine H_4 receptor is the most recently discovered member of the G protein-coupled receptor subfamily of histamine receptors. It is predominantly expressed in several cell types of immune system and in numerous areas of the CNS. Characterization of the H_4 receptor as the immune system histamine receptor with a pro-inflammatory role directed growing attention towards its therapeutic exploitation in chronic inflammatory disorders. The aim of our study was to assess the putative neuroprotective effects of the potent and selective H_4 receptor antagonist, JNJ7777120, chronically administered (1 mg/kg, i.p., twice/day for 7 days) on damage parameters in a model of focal ischemia induced in the rat by the transient (1 h) occlusion of the medial cerebral artery (tMCAo) by the monofilament technique. JNJ7777120, significantly protected from the neurological deficit 1 day after tMCAo (score at 1 day: 5.50 ± 0.43, $n = 6$ versus 9.33 ± 0.88, $n = 6$ in vehicle group; $p < 0.001$). Chronic treatment with JNJ7777120 significantly reduced body weight loss at 5 and 7 days after tMCAo compared to vehicle-treated rats ($p < 0.001$). Seven days after the ischemic insult, JNJ7777120, significantly reduced the volume of the ischemic cortical damage (20.5 ± 2.5 mm^3, $n = 6$ versus 28.3 ± 1.7 mm^3, $n = 6$ in vehicle group; $p < 0.03$) and the volume of the ischemic striatal damage (4.7 ± 0.7 mm^3, $n = 6$ versus 10.75 ± 1.9 mm^3, $n = 6$ in vehicle group; $p < 0.015$). JNJ7777120 did not significantly reduce plasma levels of the proinflammatory cytokines, IL-1β and TNF-α. Results indicate that the selective antagonist of histamine H_4 receptor, JNJ7777120, systemically and chronically administered after ischemia, reduces the ischemic brain damage and improves the neurological deficit. This study was supported by grants from the Ente Cassa di Risparmio of Florence, Italy.

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EFFECTS OF SELECTIVE HISTAMINE H_4 RECEPTOR LIGANDS ON CROTON OIL-INDUCED SKIN INFLAMMATION AND PRUITUS IN MICE

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In the present study, some structurally related ligands characterized for their different H_4R efficacies (partial agonism, neutral antagonism, partial inverse agonism, and inverse agonism) were tested in vivo against croton oil (CO)-induced ear edema and pruritus in mice. Ear weight and histological tissue damage were investigated 2 h after CO application and pruritus was evaluated 1 h after. The H_4R ligands (10–100 mg/kg) were administered subcutaneously (sc) immediately before the topical application of the irritant agent. Among the compounds tested, the neutral antagonist ST-994 and the hH_4R inverse agonist ST-1012 ($E_{max} = -110.7$ %) induced at 30 mg/kg a 46.36 and 35.16 % inhibition of ear edema, respectively, while the partial agonist ST-1006 ($E_{max} = 28.4$ %) and the partial inverse agonist ST-1124 ($E_{max} = -73.6$ %) were ineffective. CO induced a significant increase in dermis thickness, edema and leukocyte infiltration into perivascular spaces and in the interstitium. At the anti-inflammatory dose of 30 mg/kg, ST-994, but not ST-1012, significantly reduced the total severity score in all the areas examined and the number of eosinophils in inflamed tissue (60.57 % inhibition), while the total number of mast cells was not significantly modified. Ear instillation of CO induced scratching in all treated mice. CO-induced pruritus was not changed by ST-1012 at 30 mg/kg, whereas it was significantly reduced by ST-1006, ST-1124 or ST-994 (64.10, 53.62 and 65.91 % inhibition, respectively). Taken together, these data indicate that CO-induced ear inflammation and pruritus seem to be variably affected by the H_4R ligands tested. The potential advantage of the dual effect of the hH_4R neutral antagonist ST-994 in this chemically-induced skin inflammation model needs additional studies.

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ANALYSIS OF THE FUNCTION OF THE HISTAMINE H_4-RECEPTORS ON MURINE TYPE 2 INNATE LYMPHOID CELLS (ILC2) ACTIVITY

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In an asthmatic inflammatory response, the regulation of the recruitment, activation, growth, differentiation and survival of eosinophils by interleukin (IL)-5 plays a key role. Consequently, IL-5 antibodies can be considered useful for the treatment of asthma patients to prevent the recruitment of eosinophils causing local inflammation in the lung. Ovalbumin (OVA)-induced experimental allergic asthma in mice provides a good model to study the asthmatic inflammatory response. Recently, we demonstrated that the production of IL-5 in the effector phase is dependent on the expression of the histamine H_4-receptor (H_4R). However, the cell type responsible for the H_4R-regulated local production of IL-5 remained elusive. The recently identified type 2 innate lymphoid cells (ILC2) are a major source for IL-5. Therefore, we asked whether ILC2 produce IL-5 in a histamine/H_4R-dependent manner. In inflammation the activity of ILC2 is regulated by cytokines (IL-25, IL-33, TSLP) originating from epithelial cells. Thus, the H_4R-mediated regulation of ILC2 may be direct or indirect. In order to analyze a possible regulation of ILC2 by histamine, we established an in vitro assay. ILC2 and epithelial cells were isolated from mouse intestine and lung. In the obtained cells the expression of H_4R was analyzed by specific RT-qPCR. Functionally, ILC2 and epithelial cells were activated in vitro and treated with histamine or H_4R-selective agonists in the absence or presence of H_4R-selective antagonists, and IL-5 secretion (in the supernatants) were quantified by ELISA. Our data indicate that histamine via the H_4R directly modulates the induced production of IL-5. Our preliminary data show that histamine decreased IL-5 secretion ($n = 2$, $p < 0.01$ Student's T-test, two-tailed). Whether an indirect modulation also takes place is matter of ongoing investigations. This study provides evidence that the in vivo effect of the H_4R on IL-5 production in the in vivo asthma model may be based on H_4R function on ILC2 activation. This, however, has to be proved in our future studies.

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JNJ39758979 PREVENTS THE PROGRESSION OF DIABETIC NEPHROPATHY

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Due to the paucity of effective therapy, diabetic nephropathy is one of the major clinical and public health challenges, for which the pursuit of a new therapeutic strategy is a priority. Our previous data have identified the H_4R as a promising pharmacological target within this context. The aim of this study was to evaluate the efficacy of the selective H_4R antagonist JNJ39758979 (JNJ) on preservation of renal function and morphology in a murine model of diabetic nephropathy. Hyperglycaemia was

induced in DBA/2J 7–8 week-old male mice by using the multiple low-dose streptozotocyn (STZ)-injection protocol. JNJ was administered daily by oral gavage at doses of 25, 50, 100 mg/kg. Urine was processed fortnightly for standard urinalysis. At week 15 mice were sacrificed and blood and kidneys were collected for biochemical and morphological analysis. The glycaemic level rose by greater than 200 mg/dl within 14 days and remained severely elevated throughout the observation period. Despite the increase in food consumption, the weight gain of STZ-mice was significantly reduced over time ($P < 0.05$ vs control). JNJ did not significantly affect either glycaemic status or body weight, but showed a trend in reducing STZ-induced water intake. Urine collection suggested a dose-dependent inhibitory effect of JNJ on the urine volume excreted in a 24 h sample period, with animals treated for 15 weeks at 100 mg/kg excreting 10 ml/24 h urine vs 28 ml/24 h ($P < 0.05$) by animals treated with STZ alone. In addition, a significant increase in proteinuria and albuminuria was observed in diabetic mice over time. JNJ at 50 and 100 mg/kg significantly reduced proteinuria (by 58 and 43 %, respectively), with the highest dose completely effective also on albuminuria ($P < 0.05$). These beneficial effects of JNJ were confirmed by morphological analysis. JNJ was effective in preserving the renal function and morphological integrity. Thereby, our data provide the first evidence supporting the use of JNJ to counteract diabetic nephropathy.

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Molecular and genetic aspects and adverse reactions of histamine, its receptors and antihistamines

RESIDENCE TIME OF ANTIHISTAMINES AND ITS EFFECT ON SIGNALING OF THE HISTAMINE H_1 RECEPTOR

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In the body, concentrations of (endogenous) ligands are dynamic due to processes such as absorption, distribution, metabolism and excretion (ADME). This dynamic nature makes it hard to predict the effect of an antihistamine based on just its affinity constant, which only accurately predicts receptor occupancy under stable conditions (equilibrium). The dissociation rate predicts how long a ligand resides on the receptor (residence time), which is considered to be an important metric for drug effectiveness. Based on the specific dynamics of both histamine and antihistamine concentrations, the residence time of antihistamines could imply a stronger histamine inhibition then expected based on the affinity and drug concentration of both ligands. For antihistamines, residence times are often measured using radioligand binding experiments on cell membranes against an arbitrary radioligand. In this study it was explored whether the dissociation rates of antihistamines could also be directly derived from functional experiments on whole cells in competition with histamine. Different functional experiments were evaluated for their response over time upon stimulation with histamine and antihistamines and used to determine ligand residence times. It was shown that these methods allowed the discrimination of ligands based on their residence times.

The presenter of this abstract was the runner-up of the Young Investigator Award (2016).

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EFFECT OF NARROW-BAND UVB ON UP-REGULATION OF HISTAMINE H_1 RECEPTOR GENE EXPRESSION

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Ultraviolet B (UVB) irradiation shows many biological effects based on its specific spectrum. Clinically, narrow-band (NB)-UVB at 308–313 nm has a therapeutic effect in atopic dermatitis. As allergic rhinitis (AR) shares several common pathogenic features with atopic dermatitis that are successfully treated with NB-UVB phototherapy, application of NB-UVB phototherapy for AR is expected. Histamine is a major chemical mediator of the allergic reaction; its action is mainly mediated through the activation of histamine H_1 receptor (H_1R). We demonstrated that H_1R gene expression is correlated with the nasal symptom severity in patients with pollinosis and compounds that suppress the H_1R upregulation alleviates nasal symptoms in toluene-2,4-diisocyanate (TDI)-sensitized rats (N = 4, 6 week old, male, Brown Norway rat.). We also showed that PKCδ is involved in H_1R transcription and its activation by phorbol 12-myristate 13-acetate (PMA) increased the expression of H_1R at both mRNA and protein levels in HeLa cells. Here, we investigated the effects of irradiation with 310 nm NB-UVB on PMA-induced upregulation of H_1R mRNA in HeLa cells and on the TDI-induced nasal symptoms and H_1R mRNA upregulation in TDI-sensitized rats [control = 1; TDI = 1.98, $P < 0.01$ vs control; TDI + 310 nm (200 mJ/cm^2) = 1.48; TDI + 310 nm (600 mJ/cm^2) = 1.27, $P < 0.01$ vs TDI]. Irradiation with 305 nm UVB and 310 nm NB-UVB, but not 315 nm UVB at a dose of 200 and 300 mJ/cm^2 significantly suppressed PMA-induced H_1R mRNA up-regulation. (at a dose of 200 mJ/cm^2: control = 1; PMA = 3.18; PMA + 305 nm = 0.24, PMA + 310 nm = 1.08, $P < 0.0001$ vs PMA; PMA + 315 nm = 2.56. At a dose of 300 mJ/cm^2: control = 1; PMA = 2.07; PMA + 305 nm = 0.36, PMA + 310 nm = 1.72, $P < 0.0001$ vs PMA; PMA + 315 nm = 1.91, $P < 0.05$ vs PMA). At a dose of 200 mJ/cm^2, irradiation with 310 nm NB-UVB did not induce apoptosis, although 305 nm UVB did. Irradiation with 310 nm NB-UVB at the dose of 200 mJ/cm^2 suppressed PMA-induced ERK phosphorylation, while PKCδ phosphorylation was not affected. Pretreatment with 310 nm NB-UVB significantly suppressed TDI-induced nasal symptoms and H_1R gene upregulation in TDI-sensitized rats. [control = 1; TDI = 1.98, $P < 0.01$ vs control; TDI + 310 nm (200 mJ/cm^2) = 1.48; TDI + 310 nm (600 mJ/cm^2) = 1.27, $P < 0.01$ vs TDI]. Data suggest that low dose irradiation with 310 nm NB-UVB suppressed upregulation of H_1R gene expression without inducing apoptosis, resulting in the reduction in the nasal symptoms in TDI-sensitized rats, and could be useful for AR phototherapy.

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HISTAMINE AND ITS RECEPTORS AS A MODULE OF THE BIOGENIC AMINE DISEASOME

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Biogenic amines play important roles in the most important physiological processes, from cell proliferation and differentiation to nutrition, immune response, neurobiology and reproduction. These effects are spread through a wide variety of cell-specific receptors, and cell-specific signaling and metabolic pathways. However, the biochemical events underlying these effects conforms very complex networks of interactions that are far of being completely understood in most cases. In addition, two or more biogenic amines can coexist in the same physiological scenarios maintaining cross-talk events with influence in their respective physiological functions. In this sense, histamine seems to be the most pleotropic biogenic amine maintaining biochemical and functional interactions with both growth-related polyamines and neurotransmitters in different cell models and tissues. As diseases are the consequence of a biochemical imbalance in one or more tissues, the physiological importance of these compounds and their multiple relationships must be reflected in the human *diseasome*, the scope of which is not yet known. This fact impedes development of new solutions for diagnosis, prognosis and treatment of multiple diseases involving the action of biogenic amines. This work is a further effort of our group to integrate genetic, functional and clinic information about biogenic amine-related diseases assisted by text mining and network theory-based tools with the aim of helping to advance in personalized biomedical strategies. Funded by SAF2011-26518 and AMER (MINECO), and funds from PAIDI to BIO-267. CIBERER is an initiative of ISCIII (Spain).

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TIPS FOR MODELING HISTAMINE RECEPTORS

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Histamine receptors belong to class A GPCRs, rhodopsin-type family. To date, four histamine receptors are known to be activated by the same endogenous agonist (histamine) and implicated in various diseases. From a quantitative analysis of eighteen receptors and subsequently derived 153 pairs of data, we came to the conclusion that higher sequence identity between two receptors does not guarantee lower root mean square deviations (RMSD) between their structures, especially when pair-wise sequence identity lies between 25 and 40 %. This finding is somehow not in agreement with the well accepted criterion of choosing as a template the homologous protein with the highest sequence similarity, especially if the query protein shares higher than 30 % sequence identity with the template sequence. Our findings suggest that receptors having less than 50 % sequence identity should be considered and evaluated since correlation is lacking in this range. Additionally, when testing suitability for structure-based drug design, we found that choosing the template protein based on the most similar sequence resemblance only is not justified. Performance in molecular docking may be taken into account in order to determine which of the obtained models is likely to be utilized and mainly based on the enrichment factor criterion. Correlations between enrichment factors and pair-wise sequence identity of the various models will be discussed. The H_1–H_4 receptors' models which were constructed based on the 18 templates will be described and their efficacy in discriminating between actives and non-actives was reported.

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INTERSTITIAL DELETION OF HRH1, SLCA1 AND ATG7 GENES IN A DISABLED PATIENT WITH OBSERVATION OF JUVENILE IDIOPATHIC ARTHRITIS

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3p deletion syndrome is a rare condition caused by deletion of chromosome 3p25-26. Typical clinical symptoms related to this disease are low birth rate, delayed growth, microcephaly and hypotonia. Small interstitial deletions occurring at 3p25.3 may help to define critical genes involved in 3p deletion syndrome. An 11-year-old girl was hospitalized in National Institute of Geriatrics, Rheumatology and Rehabilitation due to pain, swelling, wrist ganglions and stiffening of joints. Microcephaly, psychomotor retardation, delayed speech development were recognized. Preliminary diagnosis suggested juvenile idiopathic arthritis (JIA). The patient is also under the care of genetics clinic. Whole genome oligonucleotide microarray analysis identified small interstitial chromosome 3 deletion in the region 11,031,839-11,436,721 - 404 kb. Deletion covers three genes SLCA1, HRH1 and ATG7. JIA has multifactorial pathogenesis. Genetic factors have a big influence on risk to develop disease. ATG7 gene encodes the E1-like activating enzyme involved in the 2

ubiquitin-like systems involved in MHC-I presentation. Also, HRH1 may be potentially play a role because clinically active JRA patients show abnormal histamine-inducible T suppressor cell function, characterized by the failure of CD8+, CD28-T cells to mediate any detectable suppression. This case describes for the first time deletion of histamine H1 receptor in humans. We also describe the features not previously reported for interstitial deletions at 3p25.3.

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PARALLEL HIERARCHICAL INTRA-STRAIN DIFFERENCES BETWEEN THE SUSCEPTIBILITY TO DIABETIC NEPHROPATHY AND RENAL HISTAMINE RECEPTOR EXPRESSION

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Previously we have demonstrated the renal expression of the four histamine receptors in both humans and rodents and the upregulation of both H_4R and H_3R in the kidney of diabetic rats. However, intra-species and -strain differences have been reported for histamine receptors in other tissues. Notably, it is well known that a hierarchical susceptibility to diabetic nephropathy exists between DBA/2 C57BL/6 and Balb/c mice. The aim of this study was to evaluate the correlation between histamine receptor levels and pattern of expression with renal function in healthy and diabetic mice from different inbred strains. Kidneys from DBA/2J, C57BL/6, and Balb/c 7–8 week-old male mice were processed for histamine receptor expression analysis by immunofluorescence. Pearson correlation analysis between histamine receptor expression and glycaemic status or renal function was performed in 15-week diabetic C57BL/6 and DBA/2J mice versus respective controls. The immunofluorescence analysis demonstrated that both H_1R and H_4R but not H_3R expression parallels the hierarchical susceptibility to diabetic nephropathy between DBA/2>C57BL/6>Balb/c mice. The Pearson correlation between the histamine receptor expression and the glycaemic status or the renal functional data (urine volume, proteinuria and albuminuria) revealed for C57BL/6 a positive correlation for H_1R and H_3R and glycaemic status with an r^2 of 0.45 [95 % confidence interval (CI) 0.02–0.92; $P < 0.05$] and 0.44 (CI 0.00–0.92; $P < 0.05$), respectively. In contrast, DBA/2 mice showed a strong positive correlation for H_4R and albuminuria with an r^2 of 0.97 (CI 0.88–0.99; $P < 0.001$). These data strongly support the role of H_4R in diabetic nephropathy development. The positive correlation between H_3R and urinary volume is in keeping with its localization on the collecting duct cells. Finally, the data on glycaemic status raise the question whether histamine receptors could contribute to the glucose reabsorption mechanism.

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HISTAMINE RECEPTORS LOCALIZATION IN CILIARY BODIES, RETINAE AND OPTIC NERVE IN NEW ZEALAND WHITE RABBITS

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Glaucoma is a group of eye diseases characterized by a steady loss of retinal ganglion cells and irreversible blindness. Elevated intraocular pressure (IOP) is the hallmark of this ocular pathology and it is associated with a dysfunction of the trabecular meshwork (TM). The reduction of IOP is considered the target for glaucoma treatment.

Significant histamine levels have been found in retina, choroid tissue, optic nerves and ocular mast cells. Histaminergic H_3 receptors have been localized in mammalian ocular structures, such as cones and rods of old world monkeys. DL-76 (0.5–1 % solution), as well as ABT239, GSK189254 and Ciproxifan (1 %), proved to be IOP lowering agents in an acute and chronic model of glaucoma in New Zealand White (NZW) rabbits, showing features similar to a reference anti-glaucomatous drug.

The aim of this study was to evaluate the expression and localization of histamine receptors (HRs) in ciliary body, retinae, optic nerve and TM cells derived from ciliary body to better understand the role of the histaminergic system in the eye.

We investigated the expression of HRs in ciliary bodies, retinae and optic nerve by Western blot analysis on homogenized tissue samples, immunofluorescence staining on polarized histological slides and in primary trabecular meshwork TM cell culture.

Western blot analysis demonstrated the presence of histamine H_1, H_3 and H_4 receptors in ciliary bodies, retinae and optic nerve, while H_2R was not detected. Rabbit stomach samples were used as a positive control for H_2R. The immunofluorescence staining revealed H_1R, H_3R and H_4R localization in ciliary bodies, especially on the endothelium and retinae. Moreover, the expression of these receptors was detected in TM cells by immunostaining.

These results provide evidence that HRs could represent a novel target for the development of a new class of drugs for the treatment of inflammatory and hypertensive ocular diseases.
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ARE PHENOTHIAZINE-RELATED ADVERSE DRUG REACTIONS (ADRs) COMPARABLE WITH ANTIHISTAMINE-RELATED ADRs?

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New phenothiazine derivatives with antihistaminic effects were developed in Paris in the 1940s. In the following years, phenothiazines were proven to exert an antagonistic effect not only at the histamine H_1-receptor level but also at dopamine D_1–D_5, serotonin $5HT_{2A}$, adrenergic α_1 and muscarinic M_1-receptor levels. This study aims to define whether phenothiazine-related adverse drug reactions (ADRs) are comparable with first generation anti-H_1-related ADRs. The European Database of Suspected Drug Adverse Reaction Reports was consulted in order to perform the study and all types of active phenothiazine and antihistamine molecules available in the European market were investigated.

We found that phenothiazine-neuroleptics and first generation-anti-H_1 ADRs showed similar age and gender trends. Phenothiazine ADRs for females account for 50.9 % of cases and male reactions for 45.4 %. In 5.5 % of cases, sex is not reported. In all the samples, the most involved age group is “adults between 18–64” followed by the “65–85 years old” group. Moreover, first generation neuroleptics presented a high percentage of nervous system ADRs whilst psychiatric ADRs accounted for the most common antiallergic medication ADRs.

We can conclude that, in the daily clinical use of all of these drugs, we have to consider the possible ADRs that may affect the Central Nervous System. Moreover, in all the groups a not-negligible percentage of ADRs is related to potentially serious effects such as cardiac disorders.
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ARE FIRST AND SECOND-THIRD GENERATION ANTI-H_1 ADVERSE DRUG REACTIONS (ADRs) COMPARABLE?

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First generation antihistamines are highly lipophilic and readily cross the blood–brain barrier thereby contributing to adverse central nervous system effects. In contrast, second-third generation antihistamines have a higher specificity for binding to H_1 receptors, a lower affinity for non-histamine receptors and are lipophobic with a corresponding poorer penetration of the blood–brain barrier. The aim of this study is to demonstrate whether trends in anti-H_1 ADRs involving System Organ Classes (SOCs), age group and sex are the same between first generation and second-third generation antihistamines. We accessed data from ADR Reports, the European Medicines Agency (EMA) and the European Union Drug Regulating Authority (EUDRA) joint website. We found 96 antihistaminic drugs and associations which are available in the European drug market. Of these, 73 are first generation anti-H_1 drugs and associations while 23 are second-third generation. The total number of ADRs from this pool of drugs is 89,923 for a total of 38,064 patients. Female patients represent the majority (56.2 % in both groups). Similar considerations apply to age groups where adults between 18–64 years are the most represented (63.9 vs 47.5 %). Among SOCs, we see a similar trend especially regarding the predominance of nervous system and psychiatric disorders (27.6 % vs 19.8 %). Other SOCs involved in the first generation anti-H_1 group ADRs are “injury, poisoning and procedural conditions” (15.7 %) and “general disorders and administration site conditions” (10.7 %). In second-third generation anti-H_1 ADRs, other relevant SOCs involved are “general disorders and administration site conditions” (11.7 %) and “skin and subcutaneous tissue disorders” (8.9 %). In conclusion, ADR analysis in both groups shows the same trends regarding age, sex and predominance of nervous and psychiatric disorders, suggesting an important action of second-third generation antihistamines on the central nervous system.
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Histamine in cancer, cardiovascular, endocrine and gastro-intestinal systems

PHOSPHO-Src PROTEIN MEDIATES RADIO-INDUCED MESENCHYMAL CHANGES IN MCF-7 CELLS: MODULATION BY HISTAMINE

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Radiotherapy is a prime option for treatment of solid tumors though an increase in invasiveness of neoplastic cells has been described and associated with induction of epithelial to mesenchymal transition (EMT). Our previous findings show that histamine may reduce the manifestation of mesenchymal features in irradiated MCF-7 and MDA-MB-231 breast cancer cells. We also determined that phosphorylation/activation of Src kinase is involved in histamine modulated MDA-MB-231 cell migration and invasion. The aim of this work was to investigate the role of phospho-Src (P-Src) in irradiated MCF-7 cells in relation to the invasive phenotype when cells were previously treated with histamine. Cells were treated with 20 μM histamine, 10 μM PP2 (Src inhibitor) or histamine + PP2 for 24 h, and then were irradiated with a dose of 2 Gray which is comparable to that used in fractionation radiotherapy. P-Src levels were determined immediately after irradiation while experiments to evaluate EMT molecular markers and cell migration were performed 5 days post-irradiation. Immunoblots show that ionizing radiation produced an increase in P-Src protein level ($p < 0.05$, one way ANOVA and Bonferroni). Moreover, histamine, PP2 or histamine + PP2 reduced P-Src levels ($p < 0.05$). Immunofluorescence studies show E-cadherin and Beta-catenin mainly in the cytoplasm of irradiated cells while membrane-localized E-cadherin and Beta-catenin were observed when MCF-7 cells were treated with histamine, PP2 or the combined treatment 24 h prior to gamma irradiation. The migratory capacity was studied using transwell units. Results were in agreement with P-Src levels determined above supporting the role of this activated kinase in irradiated MCF-7 cell migration and its modulation by histamine ($p < 0.05$). The association between histamine and ionizing radiation may be a challenge to further investigate and identify new targets with therapeutic potential to prevent the invasive phenotype in tumors and also enhance radiotherapy effectiveness.

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HISTAMINE POTENTIATES IONIZING RADIATION-INDUCED DNA DAMAGE IN VITRO AND IN VIVO IN HUMAN TRIPLE NEGATIVE BREAST CANCER

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We previously demonstrated that histamine selectively increases the radiosensitivity of different human cancer cell lines (MDA-MB-231, MCF-7, 1205Lu). The aims of this work were to investigate the combined effect of histamine and gamma radiation in vitro on markers of DNA damage and activity of antioxidant enzymes of two triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and MDA-BrM2) and to evaluate the histamine-induced radiosensitization in vivo in MDA-MB-231 TNBC model. Results demonstrate that histamine enhanced radiosensitivity of MDA-BrM2 cells, which display increased brain metastatic activity, mainly through the H_1R as previously described for MDA-MB-231 cells. In addition, histamine increased radiation (2 Gy dose)-induced genotoxic activity in both cell lines as evidenced by an enhanced number of γH2AX foci (marker of DNA double-strand breaks) per cell (4.5 ± 0.9 in control, 23.3 ± 1.7 in 2 Gy-untreated and 33.1 ± 1.9 in 2 Gy-histamine, $P < 0.01$ in MDA-BrM2 cells and 13.3 ± 1.4 in control, 33.0 ± 1.9 in 2 Gy-untreated and 41.1 ± 1.7 in 2 Gy-histamine, $P < 0.05$ in MDA-MB-231 cells) and also an increased 8-OHdG formation (marker of oxidative DNA damage) (one way ANOVA and Newman–Keuls tests). These effects were associated with the increased production of reactive oxygen species and the modulation of activity of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase). Histamine was also able to potentiate in vivo the anti-tumoral effect of radiation. Radiation administered as three doses of 2 Gy in consecutive days showed only modest non-significant reduction of tumor size while histamine treatment (injected sc from 1 day before irradiation until the end of the experiment) produced a 2-fold decrease in size ($P < 0.05$) and increased exponential doubling time of irradiated tumors. We conclude that histamine increases the response of TNBC cells to radiation, suggesting that it could be used as a potential adjuvant to enhance the efficacy of radiotherapy.

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HISTAMINE MODULATES TUMOUR GROWTH AND ANTITUMOUR IMMUNITY IN 4T1 TRIPLE NEGATIVE BREAST CANCER DEVELOPED IN H_4R KNOCK-OUT (H_4R KO) AND WILD-TYPE (WT) BALB/c MICE

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It is well-known that histamine regulates cellular immune responses, affecting maturation and activity of lymphocytes and myeloid-derived suppressor cells, which are considered as potential effectors of antitumor immunity and may interfere with tumor progression. The aim of the present work was to investigate the influence of the lack of histamine H_4 receptor (H_4R) on the tumorigenic capacity, metastatic potential and antitumor immunity in response to histamine in a triple negative breast cancer (TNBC) model. Thus, we evaluated tumor growth parameters, the phenotype of splenic and intra-tumoral lymphocytes in syngeneic H_4R knock-out (H_4R KO) and wild-type (WT) mice, inoculated orthotopically with 4T1 murine TNBC cells. CD4+, CD8+, CD11b+, C19+, CD3+, NK1.1+ cells were quantified in the spleens and tumors by flow cytometry. Results indicate that histamine (0.1–10 μM) produced a 2-fold decrease in proliferation evaluated by BrdU-incorporation without inducing significant apoptosis in vitro in 4T1 TNBC cells. We observed non-significant differences in latency period or tumor growth between WT and KO mice while spleen weight was significantly higher in WT than in KO mice. Histamine (1 mg/kg.day s.c.) reduced tumor volume in KO mice (743 vs. 1045 mm3 after 15 days of treatment, $^*P < 0.05$, ANOVA and Newman–Keuls post-test). In conclusion, the present study demonstrates that histamine regulates the growth of TNBC and the modulation of tumor immunity seems to be involved in histamine effects.

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HISTAMINE REDUCES THE INCIDENCE OF SEVERE MUCOSITIS INDUCED BY BORON NEUTRON CAPTURE THERAPY (BNCT) IN FIELD CANCERIZED TISSUE IN THE HAMSTER CHEEK POUCH ORAL PRECANCER MODEL WITHOUT COMPROMISING THERAPEUTIC EFFICACY

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BNCT is based on the capture reaction between boron-10, selectively targeted to tumor tissue, and thermal neutrons which gives rise to lethal, short-range high linear energy transfer particles that selectively damage tumor tissue. We demonstrated that the double application of BNCT mediated by the boron compounds BPA + GB-10 [*D*(GB-10 + BPA)-BNCT], 4 weeks between applications, 10 Gy total absorbed dose, had the best therapeutic effect in the hamster cheek pouch oral precancer model but induced severe mucositis in field cancerized tissue. In a clinical scenario, the reduction of oral mucositis is an unmet medical need, limiting tumor dose and affecting patientś quality of life. We previously demonstrated for the first time the radioprotective effect of histamine in BNCT treated animals, employing BPA, without compromising BNCT therapeutic effect. The aim of the present study was to evaluate the effect of histamine in *D*(GB-10 + BPA)-BNCT treated animals employing 2 protocols. DMBA-cancerized hamsters were treated with: (1) BNCT without Histamine; (2) BNCT + Histamine LOW concentration (1 mg/kg/day)–LONG administration (16 days); (3) BNCT + Histamine HIGH concentration (5 mg/kg/day)-SHORT administration (5 days). We evaluated histamine toxicity, % of animals with severe mucositis and % of animals with new tumors after BNCT, up to 3 months after the first irradiation at RA3 nuclear reactor. At the last time-point, the percentage of animals with severe mucositis and new tumors were analyzed using a contingency table and Fisher's exact test ($p < 0.05$). Local irritation was seen at the site of histamine injection. Protocols 2 and 3 exhibited a trend (albeit not statistically significant) to reduce severe mucositis versus group 1 (Respectively: 29 %; 20 %;

33 %), without compromising therapeutic effect vs group 1 (p = 1.0000). Protocol 3 showed a tendency to have a greater protector effect than protocol 2. Histamine HIGH concentration-SHORT administration could be more effective to reduce the incidence of severe mucositis in field cancerized tissue treated with *D*(GB-10 + BPA)-BNCT. Furthermore, this protocol involves fewer applications, reducing animal handling and the local irritation caused by the injection.

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SERUM HISTAMINE AND CYTOKINE LEVELS IN CANCER PATIENTS UNDERGOING DESENSITIZATION TO CARBOPLATIN: A PILOT STUDY

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The wide exploitation of platinum-containing chemotherapeutic agents in malignancies is coupled with increasing incidence of unforeseen hypersensitivity reactions. These potentially fatal complications have been associated with histamine-mediated type I and/or T cell-mediated type IV responses. However, the underlying mechanisms remain elusive, while desensitization is the only management option when replacement agents are related with poor prognosis. This pilot study aimed to explore the contribution of circulating histamine, TNF-α and IL-6 in IgE-mediated reaction to carboplatin. Three selected cancer patients (1 female; 2 male, 64–68 years of age) with a well-documented reaction to carboplatin were offered carboplatin skin testing and received scheduled chemotherapy cycles with desensitization sessions. Two of them had a hypersensitivity reaction during at least one desensitization session. A healthy volunteer undergoing ranitidine desensitization served as control. Serum histamine levels were quantified fluorophotometrically and TNF-α and IL-6 were determined by ELISA in all subjects. Basal serum histamine and IL-6 levels were higher, while TNF-α levels were lower in cancer patients compared to the control. Interestingly, serum histamine levels increased after ranitidine desensitization but remained unchanged during desensitization to carboplatin. Conversely, TNF-α levels significantly increased ($p < 0.05$) in carboplatin but not in ranitidine desensitization, while IL-6 levels showed inconclusive variations. Despite the small sample size, the consistency of the findings suggests that the implication of circulating inflammatory mediators in drug allergy and in the desensitization process may depend on the medication and/or on the disease pathophysiology. Furthermore, they support the need for a more systematic and careful investigation of specific symptomatic and/or aetiologic mechanisms underlying drug hypersensitivity reactions. MG received a grant from COST BM1007 to perform a STSM at NKUA.

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CARDIAC H_2 RECEPTOR OVEREXPRESSION PROTECTIVE AGAINST PP2A MEDIATED HEART FAILURE

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We have generated mice which overexpress the catalytic subunit of protein phosphatase 2A, a serine/threonine phosphatase exclusively in the heart by use of the myosin heavy chain promoter (PP2A). This led to increase PP2A enzyme activity and dephosphorylation of cardiac regulatory proteins, for instance, phospholamban and troponin inhibitor. Moreover, PP2A overexpressing mice exhibited a time dependent cardiac hypertrophy. On the other hand, histamine can exert positive inotropic and chronotropic effects in humans via H_2 histamine receptors. We have also generated transgenic mice (H_2) which overexpress the human H_2 receptor in specifically in cardiomyocytes via the alpha MHC promoter. In these mice but not in wildtype mice (WT) histamine induced increases in heart rate and ejection fraction. We crossed these mice with mice overexpressing the catalytic subunit of protein phosphatase 2A (PP2A) leading the double transgenic mice ($H_2 \times$ PP2A = DT). After about 240 days we noted reduced left ventricular ejection fraction (EF) in PP2A (33.14 ± 3.54 %, n = 16) compared to WT (54.41 ± 4.46 %, n = 10) and H_2 (59.81 ± 2.88 %, n = 10). Interestingly, in DT (43.85 ± 4.87 %, n = 11) EF was higher than in PP2A, despite similar heart rates. É/Á was elevated in DT compared to WT. Relative heart weight was

unchanged between these groups. We tentatively conclude that the histamine H_2 receptor is able to ameliorate systolic but not diastolic cardiac function of PP2A mice.

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DIAMINE OXIDASE AND MONOAMINE OXIDASE CAN DEGRADE HISTAMINE IN THE MAMMALIAN HEART TO AN INOTROPICALLY-RELEVANT EXTENT

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Histamine is metabolized by several enzymes in vitro and in vivo. The relevance of this metabolism in the mammalian heart in vivo is unclear. However, histamine can exert positive inotropic effects (PIE) and positive chronotropic effects (PCE) in humans via H_2 histamine receptors. We have generated transgenic mice (TG) which overexpress the human H_2 receptor specifically in cardiomyocytes via the α-myosin heavy chain promoter. In these mice, but not in wild type mice (WT), histamine induced PIE and PCE in isolated left or right atrial preparations. This mouse model was used to generate data on the relevance of histamine-degrading enzymes in the heart. Interestingly, the inhibitor of histamine oxidation, aminoguanidine (1 mM), shifted the log concentration response curves for the PIE to the left from $EC_{50} = 110$ nM to 37 nM ($n = 4$–6; $p < 0.05$). Furthermore, the unspecific inhibitor of monoamine oxidase, tranylcypromine (10 μM), shifted the PIE of histamine from $EC_{50} = 70$ nM to 38 nM and increased the efficacy of histamine for the PIE from a maximum response of 9.3 ± 0.8–12.4 ± 0.2 mN ($n = 3$–4; $p < 0.05$). These data indicate that exogenously applied histamine is subject to degradation in the mammalian heart by two different pathways namely diamine oxidase and monoamine oxidase. It is conceivable that drugs that inhibit these enzymes may alter cardiac function in the human heart for example via enhanced effects of histamine.

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A NOVEL SIGNALING PATHWAY CONNECTING THYROID HORMONE DERIVATIVES WITH THE HISTAMINERGIC SYSTEM

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We previously reported that 3-iodothyroacetic acid (TA1), one of the byproducts of thyroid hormone metabolism, is endowed with rapid, non-genomic, pharmacological effects including stimulation of learning, hyperalgesia and an increase in plasma glycaemia. Interestingly, these effects were also shared by 3-iodothyronamine (TIAM, the trace amine generating TA1), and were dependent on histamine release, thus allowing us to assume that T1AM and TA1 behave as endogenous neuromodulators of the histaminergic system. Currently, the clinical impact of such evidence is largely unknown. However, the clinical manifestations of thyroid diseases include itch, altered pain sensation to thermal stimuli and disturbance in the sleep/awake cycle. Exploring the hypothesis that accumulation of TA1 might be involved in such effects, we aimed to verify whether TA1 might induce pruritus, increased mice awaking time and modify the threshold to noxious heat stimuli. Itch was evaluated after s.c. administration of saline or TA1 (0.4, 1.32 and 4 $\mu g\ kg^{-1}$) in the absence or in the presence of pyrilamine (10 $mg\ kg^{-1}$; s.c.). The threshold of mice to noxious heat temperature (NHT) was evaluated by the increasing-temperature hot plate method (from 45.5 to 49.5 °C) 15 min after i.p. injection of TA1 (0.4, 1.32 and 4 $\mu g\ kg^{-1}$). Spontaneous and exploratory activities were evaluated by the hole-board test 10 min after i.p. injection of TA1 (0.4, 1.32, 4 $\mu g\ kg^{-1}$). Itch and NHT were also measured in $HDC^{+}/^{+}$ and $HDC^{-}/^{-}$ mice following injection of saline or TA1 (1.32, 4 and 11 $\mu g\ kg^{-1}$; i.p.). The awaking effect of TA1 was evaluated in mice receiving a single hypnotic dose of ethanol (3.5 $g\ kg^{-1}$, i.p.). At this setting, the time latency of the onset of sedation (LORR latency) and recovery from it (LORR duration) were measured. TA1 (0.4 and 1.32 $\mu g\ kg^{-1}$) induced itch and reduced the NHT. These effects were prevented in mice pre-treated with pyrilamine and were absent in $HDC^{-}/^{-}$ mice. TA1 (4 $\mu g\ kg^{-1}$; i.p.) increased spontaneous motility of mice and significantly increased LORR latency while reducing LORR duration. Our results confirm that TA1 pharmacologically injected into mice induces peripheral and central histamine-related effects, reinforcing the evidence of the existence of a novel

endogenous signaling connecting the thyroid with the histaminergic system.

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EFFECTS OF OLEOYLETHANOLAMIDE SUB-CHRONIC TREATMENT ON FOOD INTAKE, BODY WEIGHT AND GUT MICROBIOTA COMPOSITION IN NORMAL AND HISTAMINE DEFICIENT MICE: A PRELIMINARY STUDY

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Energy balance is an equilibrium between the amount of energy extracted from the diet and the amount expended and is regulated by a complex interplay between central and peripheral factors. We demonstrated that the fat-derived satiety factor oleoylethanolamide (OEA) requires the integrity of the central histaminergic system to fully exert its hypophagic action. Recent evidence suggests that the gut microbiota play a role in energy harvest, storage, and expenditure and its composition was shown to differ in lean and obese humans and animals and to change rapidly in response to dietary factors. Here we evaluate the effects of OEA sub-chronic treatment on food intake, body weight and gut microbiota composition as well as investigate the role of the histaminergic system in such effects. Histidine decarboxylase knock out (HDC-KO) mice and wild type (WT) littermates received daily i.p. injections of OEA (10 mg/kg, n = 4–5) or vehicle (PEG:Tween80:saline 5/5/90, n = 4–5) for 11 days, 1 h before dark onset. Faecal samples were collected after the 1st, 4th and 11th treatment day. DNAs were extracted and sequenced on a Roche 454 GS FLX+ platform for the analysis of the V3–V5 region of the bacterial 16SrRNA gene. Food consumed 1 h after OEA treatment and cumulative food consumption at the end of treatment were significantly reduced in WT mice, with respect to vehicle-treated controls, but not in HDC-KO mice. A significant reduction in body weight was also observed in OEA-treated WT mice with respect to vehicle-treated animals, while no significant changes were observed in OEA-treated HDC-KO mice with respect to controls. In agreement with the decrease of weight, an enrichment of the phylum Bacteroidetes and a reduction of Firmicutes emerged in the group of OEA-treated WT mice. This trend in terms of phyla, is associable to the appearance of a “lean” microbiota instead of an “obese”, where the trends of the two phyla are reversed. We acknowledge the Joint Programming Initiative—A Healthy Diet for a Healthy Life for support of AMBROSIAC project.

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HISTAMINE IN PROBIOTIC YOGURTS

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Probiotic yogurts are produced using cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria which evoke the fermentation of milk. In addition, they contain live micro-organisms which confer a health benefit on the host, added during or after culturing yogurt. Since all these bacteria may express histidine decarboxylase activity, the aim of this study was to examine histamine concentration in probiotic yogurts. Histamine levels and the ability to synthesis histamine by bacteria present in five commercially available in Poland probiotic yogurts at the beginning and the end of their shelf life were measured using ELISA kits (Beckman Coulter) and a microplate spectrophotometer (Eon^{TM}, BioTek). Histamine concentrations at the end of the shelf life ranged from 0.570 ± 0.05 to 1.101 ± 0.03 μg/ml and were significantly higher ($p < 0.05$, paired Student’s t-test) than at the beginning of their shelf life (0.413 ± 0.01–1.019 ± 0.03 μg/ml). Interestingly, histamine levels in cultures with L-histidine (4 g/l) supplementation were 9–10 times higher ($p < 0.001$, paired Student’s t-test) in comparison to controls (MRS Broth) without L-histidine supplementation. Therefore: (1) histamine concentration in probiotic yogurts increases with the storage time and (2) the addition of probiotic yogurts to food products containing high concentrations of L-histidine may result in consumption of large amounts of histamine.

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