Department of Pharmacology and Experimental Toxicology
Faculty of Medicine, University of Ljubljana

Inštitut za farmakologijo in eksperimentalno toksikologijo,
Medicinska fakulteta, Univerza v Ljubljani

34th Meeting of European Histamine Research Society
34. kongres evropskega združenja za raziskave histamina

Programme and Abstracts

Program in povzetki

May 11 – 14, 2005
11. – 14. maj 2005

Bled, Slovenia
Bled, Slovenija

Organizing Committee:
Lovro Stanovnik (president)
Mojca Kržan (secretary)
Tatjana Irman-Florjanc
Madeleine Ennis
Marija Čarman-Kržan
Ladko Korošec
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Poster Jury:
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Faculty of Medicine, University of Ljubljana, Slovenian Research Agency

11th May 2005

Dear Histaminologists,

welcome to the 34th Annual Meeting of the European Histamine Research Society in Bled. Many of you have visited Bled on the occasion of the 1982 meeting.

Bled is quite an attractive town at the eastern border of the Triglav National Park, around a glacial lake with a small island, not far from Ljubljana, the capital of Slovenia. From the hotel you can see the peaks of the Eastern Julian Alps and of the Karavanke mountains.

During our excursion, we will go along the southern borders of the Triglav national park. We will visit the picturesque valley of the river Soča where fierce battles were fought during World War I and which now offers various kinds of leisure activities.

We are working in quite diverse fields of biomedical research with one common point: histamine. During this meeting, 34th in the series, we will discuss the new developments in the field. The abstracts you sent us are promising an attractive scientific program consisting of oral and poster presentations. Both forms of presentation should be considered equally important; authors' preferences have been approved by the abstract evaluation committee. Brief presentations in front of the posters are expected. Main lectures also cover some interesting aspects of histamine research. One of our symposia is dedicated to Prof. Franc Erjavec, honorary member of our society, scientist and friend who recently deceased but left remarkable traces in our field and society.

We will do our best to continue the successes of the Annual Meetings of the EHRS.

On behalf of the Organising committee

Lovro Stanovnik

11. maj 2005

Dragi histaminologi,

dobrodošli na 34. letnem srečanju Evropskega združenja za raziskavo histamina, na Bledu. Mnogi od vas ste že bili tu ob našem srečanju 1982. leta.

Bled ima privlačno lego na vzhodnem robu Triglavskega narodnega parka, ob ledeniškem jezeru z otočkom, nedaleč od glavnega mesta Slovenije, Ljubljane. Iz hotela lahko vidite vrhove Vzhodnih Julijcev in Karavank.

Na izletu bomo obšli Triglavski narodni park ob njegovem južnem robu in obiskali slikovito dolino Soče, kjer so se ob med prvo svetovno vojno bile silovite bitke zdaj pa ponuja številne možnosti za razvedrilo.

Delujemo na različnih poljih biomedicinskih raziskav z eno skupno točko: histaminom. Na tem srečanju, 34. po vrsti, bomo obdelovali novosti v razvoju tega področja. Poslani povzetki obetajo zanimiv znanstven program, ki ga bodo sestavljala ustna poročila in posterji; obe obliki predstavitev imata enako težo – odbor za oceno povzetkov je upošteval želje avtorjev. Načrtujemo kratke predstavitve vsebine ob posterjih. Vabljena predavanja pokrivajo zanimive aspekte raziskav histamina. Eden od simpozijev je posvečen nedavno umrlemu prof. Francu Erjavcu, častnemu članu našega društva, znanstveniku in prijatelju, ki je zapustil pomembne sledi na raziskovalnem področju in v našem društvu.

Potrudili se bomo, da bomo nadaljevali uspehe letnih srečanj EHRS.

V imenu organizacijskega odbora

Lovro Stanovnik

PROGRAMME AT A GLANCE

WEDNESDAY, MAY 11, 2005

17:00 – 19:30 Registration

19:00 Get-Together Party

THURSDAY, MAY 12, 2005

8:30 – 9:30 Opening Ceremony

9:30 – 10:15 Invited Lecture: Gianni Marone: Superallergens: a New Mechanism of Immunologic Activation of Human Basophils and Mast Cells

10:15 – 10:45 Coffee Break

10:45 – 12:10 Prof. Franc Erjavec Memorial Symposium: Immunology and Inflammation

12:15 – 13:00 Poster Session: Immunology and Inflammation I

13:00 – 14:00 Lunch Break

14:00 – 14:45 Symposium: Cell proliferation and differentiation

14:50 – 15:35 Symposium: Metabolism and Kinetics

15:35 Coffee Break with Poster Discussion

15:40 – 16:20 Poster Session: Immunology and Inflammation II

16:25 – 17:00 Poster Session: Cell Proliferation and Differentiation

17:05 – 17:25 Poster Session: Metabolism and Kinetics

18.30 Dinner

20:15 Concert

FRIDAY, MAY 13, 2005

8:30 – 9:15 GB West Lecture: Marija Čarman-Kržan: My Journey with Histamine from the Cardiovascular System to the Brain

9:15 – 10:30 Symposium: Histamine Receptors

10:35 – 12:00 Coffee Break with Poster Discussion

10:35 – 11:00 Poster Session: Histamine receptors

11:05 – 11:35 Poster Session: Histamine and Cardiovascular and Gastrointestinal System

12:30 Excursion to Soča Valley

SATURDAY, MAY 14, 2005

9:00 – 9:45 Invited Lecture: Michael A. Beaven: The Role of Mast Cells in Allergic Disease and Innate Immunity

9:45 – 10:45 Symposium: Nervous system

10:50 – 11:15 Coffee Break

11:15 – 12:00 Poster Session: Nervous System

12:00 – 13:45 Lunch Break

14:00 – 15:15 Presentations by Young Investigators Awards Short-listed Candidates

15:15 – 15:45 Coffee Break

15:45 General Assembly

19:00 Farewell Dinner

PREGLED PROGRAMA

SREDA, 11. MAJ, 2005

17:00 – 19:30	Registracija udeležencev
19:00	Družabno srečanje ob prihodu

ČETRTEK, 12. MAJ, 2005

8:30 – 9:30	Otvoritvena slovesnost
9:30 – 10:15	Vabljeno predavanje: Gianni Marone: Superalergeni: Nov mehanizem imunološke aktivacije človeških bazofilcev in mastocitov
10:15 – 10:45	Odmor za kavo
10:45 – 12:10	Spominski simpozij prof. Franca Erjavca: Imunologija in vnetje
12:15 – 13:00	Posterska sekcija: Imunologija in vnetje I
13:00 – 14:00	Odmor za kosilo
14:00 – 14:45	Simpozij: Celična proliferacija in diferenciacija
14:50 – 15:35	Simpozij: Metabolizem in kinetika
15:35	Odmor za kavo in razpravljanje ob posterjih
15:40 – 16:20	Posterska sekcija: Imunologija in vnetje II
16:25 – 17:00	Posterska sekcija: Celična proliferacija in diferenciacija
17:05 – 17:25	Posterska sekcija: Metabolizem in kinetika
18.30	Večerja
20:15	Koncert

PETEK, 13 MAY, 2005

8:30 – 9:15	Spominsko predavanje GB Westa: Marija Čarman-Kržan: Moje potovanje s histaminom od kardiovaskularnega sistema do možganov
9:15 – 10:30	Simpozij: Receptorji za histamin
10:35 – 12:00	Odmor za kavo in razpravljanje ob posterjih
10:35 – 11:00	Posterska sekcija: Receptorji za histamin
11:05 – 11:35	Posterska sekcija: Histamin v kardiovaskularnem in prebavnem sistemu
12:30	Izlet v dolino Soče

SOBOTA, 14. MAY, 2005

9:00 – 9:45	Vabljeno predavanje: Michael A. Beaven: Vloga mastocitov pri alergijskih boleznih in prirojeni imunosti
9:45 – 10:45	Simpozij: Živčevje
10:50 – 11:15	Odmor za kavo
11:15 – 12:00	Posterska sekcija: Živčevje
12:00 – 13:45	Odmor za kosilo
14:00 – 15:15	Predstavitve izbranih kandidatov za nagrado za mlade raziskovalce
15:15 – 15:45	Odmor za kavo
15:45	Skupščina EHRS
19:00	Poslovilna večerja

PROGRAMME SCHEDULE

WEDNESDAY, MAY 11, 2005

17:00 – 19:30 Registration
19:00 Get-Together Party

THURSDAY, MAY 12, 2005

8:30 – 9:30 Opening Ceremony

Lovro Stanovnik: Opening Remarks
Madeleine Ennis: Presentation of Honorary Membership to EHRS Professor Wilfried Lorenz, introduced by Madeleine Ennis
Professor Pier Francesco Mannaioni, introduced by Emanuela Masini
Professor Takehiko Watanabe, introduced by Kazuhiko Yanai

9:30 – 10:15 **Invited Lecture** (chaired by Madeleine Ennis, Lovro Stanovnik)

Gianni Marone: Superallergens: a New Mechanism of Immunologic Activation of Human Basophils and Mast Cells (introduced by Madeleine Ennis)

10:15 – 10:45 Coffee Break

10:45 – 12:10 Prof. Franc Erjavec Memorial Symposium: Immunology and Inflammation (chaired by: Takehiko Watanabe, Andras Falus)

10:45 **Marija Čarman-Kržan**: *The Life and Work of Prof. Franc Erjavec*

10:55 **Jean Sainte-Laudy,** Ph. Belon: Use of Four Different Flow Cytometric Protocols for the Analysis of Human Basophil Activation. Application to the Study of the Biological Activity of High Dilutions of Histamine.

11:10 **Bianca Horr**, Hannelore Borck, Karen Nierich, Claudia Wackes, Friedhelm Diel. The Role of Histamine Receptor H4 in Signal Transduction of Atopic and Non-Atopic Human Lymphocytes *ex vivo.*

11:25 **Jean Sainte-Laudy**, A. Boumédiène, I. Orsel, F. Touraine, M. Cogné: Analysis of IgE Receptor Internalisation Induced by Basophil Activation. Application to the Muscle Relaxants Allergic Hypersensitivity Diagnosis by Flow Cytometry.

11:40 **Elke Schneider,** François Machavoine, Jean-Marie Pléau, Robin L. Thurmond, Hiroshi Ohtsu, Takehiko Watanabe, Alfred H. Schinkel, Michel Dy: Organic Cation Transporter 3 modulates murine basophil functions by controlling intracellular histamine levels

11:55 **Roman Khanferyan**, A. Andrejanova, D.Lesik, N. Milchenko, N. Riger: On the Role of Histamine and Different Histamine Receptors in IgE Synthesis.

12:15 – 13:00 **Poster Session: Immunology and Inflammation I**
(chaired by: Zsuzsanna Horváth and Rado Nosál)

P-01. **Adam J. Byrne**, John J. Walsh: Studies on the Mast Cell Stabilising Properties of Plant Extracts with Reputed Anti-Inflammatory Activity.

P-02. **Erna Pap**, Éva Pállinger, Dorottya Mihályi, Friedhelm Diel, and András Falus: Histamine, a Possible Mediator in Placental Cytokine Expression.

P-03. Danuta Maślińska, Milena Laure-Kamionowska, Krzysztof T. Maśliński, Elzbieta Wojtecka-Łukasik, Slawomir Maśliński, **Mariusz Gujski**: Toll-Like Receptors (TLRs) and Histamine Receptor H4 in Articular Tissues of Patients with Rheumatoid Arthritis (RA).

P-04. E. Wojtecka-Lukasik, M.A. Karolczak, D. Maslinska, **Mariusz Gujski**, K. Czuprynska, K. Gruda, S. Maśliński: Histamine and Oxygen Radicals of Blood in the Course of Cardiopulmonary Bypass (CPB).

P-05. E. Wojtecka-Lukasik, **Mariusz Gujski**, K. Czuprynska, D. Maślińska, S. Maśliński: Taurine – Chloramine is a Potent Antiinflammatory Substance.

P-06. E. Dorofeeva, **Roman Khanferyan**: The Mechanisms of Histamine Release and IgE Synthesis Induced by Trichotecenic Mycotoxins

P-07. **Zoltán Wiener**, Márton Keszei, Barbara Köhalmi, Sára Tóth, András Falus: TGF-β Modifies the Gene Expression Profile of *in vitro* Differentiated Human Mast Cells.

P-08. **Valéria László,** Ivett Jelinek, Edit Buzás, Éva Pállinger, András Falus: Increased Antigen Presentation and Th1 Polarisation in Genetically Histamine-Free Mice.

P-09. **Karen Nierich**, Bianca Horr, Hannelore Borck, Claudia Wackes, Janine Diel, Vanina Medina, Sandra Rieger, Friedhelm Diel: Signal Transduction and Activator of Transcription (STAT) is Phosphoregulated by Histamine in Human Lymphocytes ex vivo – New Aspects

13:00 – 14:00 Lunch Break

14:00 – 14:45 Symposium: Cell Proliferation and Differentiation (chaired by: Emanuela Masini, Ėva Pállinger)

14:00 **Emanuela Masini**, V. Fabbroni, L. Giannini, A. Vannacci, F. Fabrizi, C. Uliva, N. Schiavone, E. Fanti, F. Perna, C. Cortesini, F. Cianchi: Histamine Stimulates Tumor Proliferation and Angiogenesis through H2/H4 Receptor-Mediated Cyclooxygenase-2 Activation in Colorectal Cancer.

14:15 **Sára Tóth**, Elen Gócza, Bogdan V. Carstea, Barbara Uzonyi, András Falus: Comparison of the Differentiation Capacity of Histamine Deficient (HDC KO) and Wild Type Embryonic Stem (ES) Cells.

14:30 **Ėva Pállinger,** Zs. Horváth, E. Buzás, H. Hegyesi, I. Jelinek, R.L. Thurmond, A. Falus: Is There Any Influence of the Absence of H4 Receptor on T Cell Development: Comparison of T Cell Precursors and Thymic Stroma Cells Involved in Thymic Selection of H4 Receptor Knockout (H4R-KO) and Wild Type (WT) Mice.

14:50 – 15:35 **Symposium: Metabolism and Kinetics** (chaired by: Tatjana Irman-Florjanc, Hubert G. Schwelberger)

14:50 **Tatjana Irman-Florjanc:** Pharmacokinetic Effects of a Single Dose of Antidepressant on Histamine in the Rat

15:05 **Hubert G. Schwelberger**: Origins of Plasma Amine Oxidases in Different Mammalian Species.

15:20 Aurelio A. Moya-Garciá, Daniel Rodríguez-Agudo, Miguel Angel Medina, **Francisca Sánchez-Jiménez**: New Structural Insights to Search for Selective Mammalian Histidine Decarboxylase Inhibitors.

15:35 Coffee Break with Poster Discussion

15:40 – 16:20 Poster Session: Immunology and Inflammation II
(chaired by: Zoltán Wiener and Friedhelm Diel)

P-10. Viera Jančinová, Katarína Drábikova, **Rado Nosál,** Magdaléna Májeková, Dagmar Holomáňová: Antiradical Effects of Antihistamines. Structure-Activity Relationship.

P-11. M. Petríková, Viera Jančinová, **Rado Nosál,** Magdaléna Májeková, and Dagmar Holomáňová: H_1- Antihistamines and Activated Blood Platelets.

P-12. **Katarina Drábiková**, Viera Jančinová, Rado Nosál, Jana Pečivová, Tatiana Mačičková: Extra- and Intracellular Oxidant Production in Phorbol Myristate Acetate Stimulated Human Polymorphonuclear Leukocytes: Modulation by Histamine and H1-Antagonist Loratadine® .

P-13. **Luka Peternel,** Gorazd Drevenšek, Manica Černe, Mateja Štempelj, Uroš Urleb, Metka V. Budihna: Protective Effect of Clemastine against Compound 48/80-Induced Drop of Mean Arterial Blood Pressure in the Rat.

P-14. **Mateja Štempelj**, Aljoša Bavec, Ilonka Ferjan: Regulation of Nerve Growth Factor-Induced Histamine and Arachidonic Acid Release from Rat Mast Cells by Cannabinoids.

P-15. A. Vannacci, C. Marzocca, L. Giannini, L. Mazzetti, S. Franchi-Micheli, P. Failli, E. Masini, P.F. Mannaioni: Evaluation of the Effects of a Novel Carbon Monoxide Releasing Molecule (CORM-3) in an *in vitro* Model of Cardiovascular Inflammation.

P-16. **Jana Kralova**, Milan Ciz, Radomir Nosál, Katarína Drábikova, Antonin Lojek: The Effect of H1-Antihistamines on Oxidative Burst of Phagocytes.

P-17. Lynne C. Tetlow, **David E. Wooley**: Histamine and PGE2 Stimulate the Production of Interleukins-6 and –8 by human articular chodrocytes *in vitro*.

16:25 – 17:00 Poster Session: Cell Proliferation and Differentiation (chaired by: Wieslava Agnieszka Fogel and Zsuzsanna Darvas)

P-18. **Zsuzsanna Horváth,** Éva Pállinger, Győző Horváth, András Falus: Bone Marrow Regeneration and IL-3 Expression Are Delayed in Histidine Decarboxylase Knock Out (HDC-ko) Mice.

P-19. Graciela Cricco, Mariel Núñez, Vanina Medina, Gloria Garbarino, Nora Mohamad, Alicia Gutiérrez, Claudia Cocca, Carolina Kirchheimer, Rosa Bergoc, Elena Rivera, **Gabriela Martin**: Histamine Modulates Cellular Events Involved in Tumor Invasiveness in Pancreatic Carcinoma Cells.

P-20. **Peter Pócza,** Mark Keresztesi, Peter Kovács, Eva Pállinger, Laszlo Kőhidai, András Falus and Zsuzsa Darvas: Histamine Influences on the Migration, Expression of Adhesion Molecules and MMPs in Human Melanoma Cell Line

P-21. **Vanina Medina**, Bianca Horr, Hannelore Borck, Gloria Garbarino, Graciela Cricco, Karen Nierich, Claudia Wackes, Friedhelm Diel, Elena S. Rivera: The Role of STAT1 and 6 in Histamine-Mediated Growth Inhibition of Human Breast Carcinoma Cell Lines.

P-22. **Grzegorz Szewczyk,** Michal Pyzlak, Waclaw Śmiertka, Jakub Klimkiewicz, Dariusz Szukiewicz: Histamine Stimulates Differentiation of Human Trophoblast through H1 Receptor.

P-23. Dariusz Szukiewicz, **Grzegorz Szewczyk**, Jakub Klimkiewicz, Michal Pyzlak, Danuta Maslinska: The Role of Histamine and its Receptors in the Development of Ovarian Follicles *in vitro*.

P-24. Danuta Maślińska, Milena Laure-Kamionowska, Krzysztof T. Maśliński, Slawomir Maśliński, Krzysztof Deręgowski, **Grzegorz Szewczyk**: Histamine H4 Receptor on Mammary Epithelial Cells of the Human Breast with Different Types of Carcinoma.

17:05 – 17:25 Poster Session: Metabolism and Kinetics (chaired by: Anita Sydbom and Ilonka Ferjan)

P-25. **Johannes Feurle**, Simona Rajtar, Tatjana Irman-Florjanc, Hubert G. Schwelberger: Molecular Cloning of Guinea Pig Diamine Oxidase and Histamine N-Methyltransferase.

P-26. **Wieslava Agnieszka Fogel**, Andrzej Lewinski: Experimental Ulcerative Colitis in Rats; the Effect of Diamine Oxidase Administration

P-27. **Simona Rajtar**, Johannes Feurle, Hubert G. Schwelberger, Tatjana Irman-Florjanc: Expression of Histamine Degrading Enzymes in Guinea Pig Tissues.

P-28. **Andreas Sponring,** Hubert G. Schwelberger: Regulation of Diamine Oxidase Expression in Mammalian Cells.

18.30 Dinner

20:15 Concert

Friday, May 13, 2005

8:30 – 9:15 **GB West Lecture** (chaired by: Pier Francesco Mannaioni, Walter Schunack)

Marija Čarman-Kržan: My Journey with Histamine from the Cardiovascular System to the Brain (introduced by Walter Schunack)

9:15 – 10:30 Symposium: Histamine Receptors (chaired by: Pier Francesco Mannaioni, Walter Schunack)

9:15 **El-Sayed K. Assem**, Beatrice Y. Wan, Kheng H. Peh, Frederick L. Pearce: Effect of Genistein on Agonist-Induced Airway Smooth Muscle Contraction.

9:30 **Remko A. Bakker**, H.D. Lim, N. Terzioglu, R.M. van Rijn, I.J. de Esch, R. Leurs: New Ligands For The Histamine H_4 Receptor.

9:45 M. Čarman-Kržan, A. Bavec, M. Zorko, W. Schunack: Differences in the Binding Characteristics and Intracellular Effects of Specific Histamine H_1- Agonists - Pyridylbutylhistaprodifens in the Cardiovascular Tissue.

10:00 **Nicholas I. Carruthers**: Conformational Restriction in the Design of Diamine –Based Human Histamine H_3 Receptor Antagonists.

10:15 **Fiona C. Shenton,** Richard van Rijn, Rob Leurs, Remko Bakker, Paul L. Chazot: Evidence for Human and Rodent H_3 Receptor Dimers: A Cross-Linking Study.

10:35 – 12:00 Coffee Break with Poster Discussion

10:35 – 11:00 Poster Session: Histamine Receptors (chaired by: Nicholas I. Carruthers and Metoda Lipnik-Štangelj)

P-29. **Maristella Adami**, Gabriella Coruzzi, Elena Guaita, Iwan J.P. de Esch, Rob Leurs: Antiinflammatory, Analgesic and Gastroprotective Effects of the Novel and Selective Histamine H_4-Receptor Antagonist VUF5949.

P-30. Beatrice Y. Wan, Kheng H. Peh, **El-Sayed K. Assem**, Frederick L. Pearce: Cross-Interaction between Par-Activation and Tyrosine Kinase in the Modulation of Rat Ileum Smooth Muscle Contraction (RIMC).

P-31. **Marlon Cowart**, Gregory Gfesser, Kaushik Bhatia, Renee Esser, Minghua Sun, Thomas R. Miller, Kathleen Krueger, David Witte, Timothy A. Esbenshade, Arthur A. Hancock: Fluorescent Benzofuran H_3 Antagonists with Sub-Nanomolar Potency.

P-32. **Peter C. Konturek**, T. Brzozowski, P. Gaca, G. Burnat, M. Raithel, S.J. Konturek, E.G. Hahn: Histamine and its Metabolite N-alpha-methyl-histamine Accelerate Ulcer Healing in Rats: Role of H3 Receptors, Sensory Nerves and TGFα

P-33. **Enzo Poli**, Cristina Pozzoli, Gabriele Regina, Alessandro Menozzi, Luigi Roncoroni, and Gabriella Coruzzi: Dualistic H_1-Mediated Effect of Histamine on the Isolated Human Intestine.

P-34. **Arthur A. Hancock**, R. Scott Bitner, Kathleen M. Krueger, Stephani Otte, Arthur L. Nikkel, Thomas A. Fey, Eugene N. Bush, Robert W. Dickinson, Robin Shapiro, Victoria Knourek-Segel, Brian A. Droz, Michael E. Brune, Peer B. Jacobson, Marlon D. Cowart, and Timothy A. Esbenshade: Distinctions and Contradistinctions between Antiobesity Histamine H_3 Receptor (H_3R) Antagonists Compared to Cognition-Enhancing H_3 Receptor Antagonists.

11:05 – 11:35 Poster Session: Histamine and Cardiovascular and Gastrointestinal System (chaired by: Mojca Kržan and Enzo Poli)

P-35. **Jerzy Jochem**, Krystyna Zwirska-Korczala: Interactions Between the Histaminergic and Angiotensinergic Systems in the Central Cardiovascular Regulation in Rats.

P-36. Tatjana Irman-Florjanc, **Jerzy Jochem**, Krystyna Zwirska-Korczala: Influence of Centrally Acting Amitriptyline and Citalopram on Histamine-Induced Cardiovascular Effects in Rats.

P-37. **Martin Raithel,** J. Maiss, A. Nägel, A. Reissmann, J. Kressel, E.G. Hahn, P. Konturek: First Evidence of Histamine (H) Release Involved in Human Reflux Disease.

P-38. B. Backhaus, M. Weidenhiller, P. Bijlmsa, E.G. Hahn, **Martin Raithel**: Gut Mucosal Histamine Release (HR) in Response to Polyamines is Different in Patients with Colorectal Adenoma and Controls: Implications for Colorectal Adenoma Growth?

P-39. B. Backhaus, E.G. Hahn, **Martin Raithel**: Non-Immunogically Induced Histamine Release (HR) of Vital Biopsies of the Human Gut by Stimulation with Polyamines.

P-40. **Claudia Wackes**, Maria Herwald, Hannelore Borck, Eva Diel, Louisa Page, Bianca Horr, Linda Rohn, Friedhelm Diel: Histamine in Lager Beer.

12:30 Excursion to Soča Valley

Saturday, May 14, 2005

9:00 – 9:45 Invited Lecture (chaired by: Pertti Panula, Kazuhiko Yanai)

Michael A. Beaven: The Role of Mast Cells in Allergic Disease and Innate Immunity (introduced by Marija Čarman-Kržan)

9:45 – 10:45 Symposium: Nervous System (chaired by Pertti Panula, Kazuhiko Yanai)

9:45 K. Tekes, M. Hantos, B. Bizderi, M. Gyenge, V. Kecskeméti, and **Zsuzsanna Huszti**: Comparison of Nociceptin- and Compound 48/80-Induced Histamine Release After a Single, Intracerebroventricular Administration of the Compounds.

10:00 **Ilhan Celik**, Wuttipong Tirakotai, Thomas Riegel and Hans-D. Mennel: Mast Cell Density in Secretory Meningiomas.

10:15 Gabriele Cenni, Patrizio Blandina, Pier Francesco Mannaioni, and **M. Beatrice Passani:** Systemic and Local Effects of Cannabinoids on Histamine Release in the Rat Brain.

10:30 Hongmei Dai, **Kazuhiko Yanai:** Blockage of Histamine H1 Receptor Attenuates Social Isolation-Induced Disruption of Pre-Pulse Inhibition: A Study in Histamine H1 Receptor Gene Knockout Mice.

10:50 – 11:15 Coffee Break

11:15 – 12:00 Poster Session: Nervous System (chaired by: Arthur A. Hancock and Lovro Stanovnik)

P-41. **Gabriele Cenni**, M. Beatrice Passani, and Patrizio Blandina: Histamine H_3 and GABAa receptors modulate the activity of histamine neurons.

P-42. **Gabriele Cenni**, M.B. Passani, P.F. Mannaioni, M.D. Efoudebe, and P. Blandina: Effects of betahistine on ACh and histamine release from the rat brain.

P-43. **Timothy A. Esbenshade**, Marina Strakhova, Tracy L. Carr, Rahul Sharma, David G. Witte, Betty B. Yao, Thomas R. Miller, Arthur A. Hancock: Differential CNS Expression and Functional Activity of Multiple Human H_3 Receptor Isoforms.

P-44. **Timothy A. Esbenshade**, Kathleen M. Krueger, Betty B. Yao, David G. Witte, Brian R. Estvander, John L. Baranowski, Thomas R. Miller, Arthur A. Hancock: Differences In Pharmacological Properties of Histamine H_3 Receptor Agonists and Antagonists Revealed at Two Human H_3 Receptor Isoforms.

P-45. **Sotirios Kakavas**, Ekaterini Tiligada: *In Vivo* Effect of Sodium Cromoglycate on the Reduction of Hypothalamic Histamine Levels in Hyperthyroid Rats.

P-46. **Mojca Kržan**, Joan P. Schwartz: Histamine Transport in Neonatal and Adult Astrocytes.

P-47. **Metoda Lipnik-Štangelj** and Marija Čarman-Kržan: Histamine and IL-1β Interactions in PKC-Stimulated NGF Secretion from Glial Cells.

12:00 – 13:45 Lunch Break

14:00 – 15:15 Presentations by Young Investigators Awards Short-listed Candidates (chaired by: Gill Sturman and Madeleine Ennis)

14:00 **Kimmo A. Michelsen**, Adrian Lozada, Jan Kaslin, Kaj Karlstedt, Tiina-Kaisa Kukko-Lukjanov, Irma Holopainen, Hiroshi Ohtsu and Pertti Panula: Histamine-Immunoreactive Neurons in the Mouse and Rat Suprachiasmatic Nucleus.

14:15 **Zoltán Pós,** Hargita Hegyesi, András Falus: Phenotypic Profiling of Experimental Murine Melanoma Tumors with Transgenically Manipulated HDC Expression.

14:30 **CongYu Jin**, Pertti Panula: Altered H1 and H2 receptor binding in the hippocampal areas of subjects with major psychoses.

14:45 **Ivett Jelinek**, Valéria László, Éva Pállinger, Hargita Hegyesi, Robin L. Thurmond, András Falus: H4R is Implicated in Various Effects Exerted by Histamine on Mouse Dendritic Cells.

15:00 **Vanina Medina**, Graciela Cricco, Gloria Garbarino, Mariel Núñez, Gabriela Martín, Claudia Cocca, Rosa M. Bergoc, Elena S. Rivera: Differential Effects of Histamine-Mediated Signaling Processes in Normal and Malignant Human Mammary Cells.

15:15 – 15:45 Coffee Break

15:45 General Assembly

19:00 Farewell Dinner

ABSTRACTS

Superallergens: a New Mechanism of Immunologic Activation of Human Basophils and Mast Cells

Gianni Marone, Francesca W. Rossi, Bianca Liccardo, Aikaterini Detoraki, Giuseppe Spadaro
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Superantigens have the unique ability to interact specifically with most lymphocytes expressing antigen receptors from a particular variable region gene family. Classical superantigens are T-cell superantigens. Some naturally occurring proteins are superantigens for B-lymphocytes. These proteins are endowed with “unconventional” immunoglobulin-binding capacities. We conducted experiments to determine whether immunoglobulin superantigens activate human basophils and mast cells to release mediators and cytokines. Protein Fv, released in biological fluids of patients affected by viral hepatitis, stimulated histamine and cytokine release from basophils. IL-4 and IL-13 mRNA were increased after stimulation by protein Fv. Basophils from which IgE have been removed no longer released cytokines in response to protein Fv and anti-IgE. Human monoclonal IgM V_H3^+ inhibited protein Fv-induced secretion of IL-4 and histamine from basophils and lung mast cells. HIV-1 gp120 is an immunoglobulin superantigen, which might explain the activation of B-lymphocytes in patients with AIDS. gp120 from divergent HIV-1 isolates increased IL-4 and IL-13 mRNA expression parallel to histamine secretion from basophils and mast cells. gp120 activates these cells through interaction with IgE, since removal of IgE completely blocked the glycoprotein’s effects on secretion. Preincubation of gp120 with monoclonal IgM V_H3^+ also inhibited its effect on $Fc\varepsilon RI^+$ cells. *Peptostreptococcus magnus* is a bacterium expressing a cell wall protein L that binds human immunoglobulins through interaction with κ light chains. Protein L and a recombinantly expressed fragment (B1-B4) covering the immunoglobulin-binding domains induced histamine and cytokine (IL-4 and IL-13) release from basophils. Preincubation of protein L with IgE from myelomas expressing λ chains did not affect the activating property of protein L, whereas IgE from myeloma with κ chains completely blocked protein L activity. In conclusion, a novel mechanism may be envisaged by which endogenous, viral and bacterial proteins activate human $Fc\varepsilon RI^+$ cells thereby acting as superallergens.

Use of four different flow cytometric protocols for the analysis of human basophil activation. Application to the study of the biological activity of high dilutions of histamine

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Results obtained during the last ten years about the biological activity of high dilutions of histamine have been the aim of a wide controversy. For a better understanding of this phenomenon, we focused our attention on various markers of the activation cascade and set up three new flow cytometric protocols based on a tri-colours (CD13, CD63, CD203c), a quadri-colours (CD13, CD14, CD63, CD203c) and on a new bi-colours (anti-IgE/CD203c) analysis of basophil activation, the activation markers being CD63, CD203c and IgE (IgE receptor internalisation). In contrast to our previous studies, basophil activation was induced by fMLP and results were expressed in index calculated from CD63 percentage activation and CD203c/ IgE shift of the mean intensities of fluorescence.

The mean inhibition induced by histamine 16 C (12.4%,10 experiments) was not significant ($p=0.15$, Mann and Whitney) with the 3 colours protocol and was significant (39%, 5 experiments, $p= 0.005$) with the 4 colours protocol. Histamine 2C positive control was capable of inducing a significant inhibition with both protocols (respectively 73%, $p< 0.001$ and 75%, $p< 0.01$). Percentage inhibition observed with the 4 colours protocol were higher than with the anti-IgE/anti-CD63 protocol (19,4%) and reached approximately 50% of the inhibition induced by histamine 2C.

The bi-colours protocol (anti-IgE/anti-CD203c) gave the highest inhibitions reaching values observed for histamine 2C.

In addition to our previous results showing that histamine high dilutions were capable of inhibiting an IgE dependant basophil activation, these results were in favour of their capacity to inhibit also an IgE independent activation.

The magnitude of the inhibition depended on the activation marker analysed and is maximum for the earliest markers such as here the internalisation of IgE receptors.

The Role of Histamine Receptor H4 in Signal Transduction of Atopic and Non-atopic Human Lymphocytes ex vivo

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In previous work it was demonstrated that signal transduction (ST) in Th lymphocytes is related by signal transducer and activators of transcription (STAT). The objectives of this study were to investigate the role of the histamine H4-receptor on the STAT1/STAT6 responses in atopic and non-atopic lymphocytes.
PBMC of 6 atopics (IgE>1000 IU) as well as 6 matched non-atopics (IgE<100 IU) were stimulated with PHA and incubated for 3 days. Histamine (His), thioperamide (Thio), clobenpropit (Clob) and JNJ7777120 (Johnson & Johnson PRD, USA) were added alone or in combination 4 hours post-plating. Western blots were performed for determination of STAT1/STAT6. EMSA was performed for binding tests with IRDye^{TM}-labeled STAT1 oligoDNA (metabion, Germany) and measured with the Odyssey® infrared imaging system.
As we could show previously IL-4 and STAT6 were elevated in atopic blood Th2-lymphocytes. His inhibited STAT6, but this could not be influenced by the selective H4-antagonist, JNJ7777120. Surprisingly JNJ7777120 antagonized the inhibitory responses of His on STAT1α/β phosphorylation. Corresponding to this the intracellular concentration of latent STAT1 was increased. Although latent STAT1 is decreased in atopy, His had additional influence on the STAT1α/STAT1β relation during the first day of the cell culture. Thio (H3/H4-antagonist), Clob (H3-antagonist/H4-agonist) and JNJ7777120 indicated the crucial role of H4-receptor in the ST processes of blood lymphocytes as well as STAT1 DNA binding experiments with oligoDNA 5'-IRD700-CAT GTT ATG CAT ATT CCT GTA AGT GAA AA-3'.
Although the different responses in the atopic and non-atopic group remain unclear, it can be suggested that His modulates the IL-4 induced JAK/STAT pathway predominantly in the atopic cell cultures via His H4-receptor. However, STAT6 phosphorylation is regulated via different His-receptors than H4 e.g. H2.

Analysis of IgE receptor internalisation induced by basophil activation. Application to the muscle relaxants allergic hypersensitivity diagnosis by flow cytometry.

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Flow cytometric analysis of basophil activation is today one of the most promising method for the diagnosis of IgE dependant allergic hypersensitivities results obtained so far showing, for proteins, very high sensitivities and specificities. In contrast, for drugs, the picture is quite different, sensitivities published so far standing around 50%.
We checked other activation markers and particularly IgE , previous studies carried out having shown that allergen induced basophil activation was followed by a deep decrease of the anti-IgE FITC labelled basophil population mean fluorescence intensity (MFI-IgE).
We applied this method to proteins and to muscle relaxants allergy diagnosis, results being expressed in index calculated from the MFI-IgE and percentage of CD63 up-regulation. Two positive controls (anti-IgE and fMLP) were included. Height patients allergic to proteins (rubber latex, gelatin, grass pollen and peanut) and 8 patients having experienced a per-anaesthetic shock were selected these latter according to the French Society of Anaesthesia and Resuscitation (SFAR) criteria.
fMLP induced a significant activation in all cases, whereas one anti-IgE non responder was excluded of the study. For proteins, the index ranged from 15 to 2 000 000 (positivity threshold=6) with a total correlation with clinical data.
For the remaining 7 muscle relaxants sensitised patients flow cytometry was positive in all cases for at least one molecule. Correlation with skin tests was significant (khi2, $p < 0.001$).
These results confirmed that without decreasing specificity, sensitivity of flow cytometry depends of the kinetic of the activation marker analysed, and increases by the use of early markers of the activation cascade. For muscle relaxants we reached here a high sensitivity and a high specificity, both mandatory for the use of this method in allergy diagnosis.

Organic Cation Transporter 3 modulates murine basophil functions by controlling intracellular histamine levels

Elke Schneider, François Machavoine, Jean-Marie Pléau, Robin L. Thurmond, Hiroshi Ohtsu, Takehiko Watanabe, Alfred H. Schinkel and Michel Dy
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Upon stimulation, bone marrow cells of the basophil lineage are capable of producing histamine, interleukin (IL)-4 and IL-6 concomitantly. They can also take up histamine from the environment, a process that is inhibited by a number of antagonists of H_3 histamine receptors, which are not expressed in bone marrow cells. Here, we set out to characterize the protein mediating the transport of histamine in basophils and demonstrate that it is identical with Organic Cation Transporter 3 (OCT3, SLC22A3). This conclusion is supported by the following findings: 1) *Oct3* mRNA was expressed in basophil-enriched bone marrow cells. 2) Inhibitors of this transporter were as effective as H_3/H_4R ligands in competing with [^{3}H]histamine for uptake. 3) Bone marrow cells were similarly labeled by [^{3}H]histamine and the prototypical ligand of organic cation transporters [^{3}H]methyl-4-phenylpyridinium ($MPP^{+)}$. 4) Histamine uptake did no longer occur in OCT3-deficient mice. We show that, similarly to histamine itself, substrates of OCT3 inhibit IL-3-induced histamine, IL-4 and IL-6 production by basophils from wild type but not from $OCT3^{-/-}$ mice. The inhibitory effect of the drugs tested depends on intracellular histamine since it was strikingly reduced in mice in which histidine decarboxylase had been deleted, while exogenous histamine retained its activity, proving that intracellular histamine levels must reach a critical threshold to induce the negative feedback signal. We postulate that pharmacological modulation of OCT3 might become instrumental in the control of basophil functions during allergic diseases.

On the Role of Histamine and Different Histamine Receptors in IgE Synthesis

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It is well-known that histamine plays crucial role in the pathogenesis of allergic and different non-allergic diseases. Less attention paid to the immunoregulatory role of histamine. The goal of this investigation was to evaluate the role of histamine as well as different histamine receptors in the regulation of IgE synthesis. It has been shown that histamine modulate IgE in vitro synthesis differently in healthy voluntaries, allergic patients, sensitive to ragweed pollen and individuals with bone marrow alterations. Histamine-mediated effects on IgE synthesis realized through not only well-investigated H_1 and H_2 receptors, as well as via new determined and recently cloned H_3 and possibly H_4 receptors. IgE synthesis of histamine highly dependent on the concentration of mediator and type of involved receptors. Thus, histamine in high concentrations (10^{-5}M) suppressed and in low concentrations (10^{-8}M) stimulated spontaneous IgE synthesis. The study of several imidazole and non-imidazole H_3/H_4 antagonists (FUB 181, FUB 649, FUB 372, Imoproxifan-IMP and Ciproxifan -CF) showed that IgE synthesis depends on the potency and structural differences of investigated H_3/H_4 antagonists,. Thus, high concentrations of IMP and CF induce significant increase in IgE synthesis, especially in Con-A-stimulated MNC cultures. The increased synthesis was IL4-dependent. FUB 181 induced only minimal increase, while FUB 372 had no effect on IgE synthesis. At the same time FUB 849 profoundly decrease IgE response. At the same time IgE synthesis depends on the preexisting levels of IgE. Thus, FUB 181 in high concentration (10^{-5}M) increased IgE synthesis only in allergic patients with low level of IgE, but not in group having high spontaneous levels of IgE synthesis. The study showed that histamine and different types of histamine receptors are involve in IgE synthesis in allergic and non-allergic patients. IgE-modulatory activity of histamine is highly dose-dependent. The effects of new types of H_3/H_4 antagonists depends on their potency and structure.

Acknowledgment: Many thanks to prof. W. Schunack for providing us several agonists and antagonists of histamine receptors.

Studies on the mast cell stabilising properties of plant extracts with reputed anti-inflammatory activity

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Mast cells are effecter cells in IgE-associated immune responses, such as anaphylaxis, allergic rhinitis, asthma and atopic dermatitis. Mast cells have also been implicated in the aetiology of multiple sclerosis (1), inflammatory arthritis (2), cystic fibrosis (3) and tumour angiogenesis (4). Among the preformed and newly synthesised substances released on degranulation of mast cells, histamine remains the best characterised and most potent vasoactive mediator implicated in the early phase of immediate hypersensitivity reactions. Several plant extracts with reputed anti-inflammatory activity were evaluated for their ability to inhibit the release of histamine from isolated rat peritoneal mast cells. Methanol extracts (2mg/ml) of the heartwood of *Quercus petraea*, the dried stem of *Sargentodoxa cuneata* and the root of Devils claw (*Harpagophytum procumbens*) inhibited compound 48/80 induced histamine release. The inhibition values were 85.71 ± 7.20%, 86.61± 10.78% and 52.88±12% respectively. The distilled oil of Juniper berries *(Juniperus communis*) (2mg/ml of the oil) also inhibited compound 48/80 induced histamine release (91.42±4.0%). Extracts of *Q. petraea* and *S. cuneata* inhibited calcium ionophore A23187 induced histamine release. The respective inhibition values were 29.88±7.20% and 36.87±8.03%.

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Histamine, a Possible Mediator in Placental Cytokine Expression

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The role of histamine in female reproductive processes has been described from several aspects. Pre – and postimplantation events are accompanied by high HDC activity and HDC expression is much higher in the placenta than in other organs, the presence of H1 and H2 receptors have also been shown in the placenta.

Considering that Th2 dominance is crucial during pregnancy and that during inflammatory processes Th2 dominance is initiated by histamine also, our objective was to study the role of histamine in the actual tuning of T helper lymphocyte (Th1/Th2) balance for the maintenance of pregnancy at the lymphocyte and at the placenta level.

We used our HDC knockout mouse model for our experiments.To clarify the importance of the presence of histamine in the placenta, we measured its concentration by HPLC. In wild type (WT) animals it was 700 ng/ml/placenta, meanwhile there was practically no histamine in knockout (KO) animals' placentae. The spleen of non-pregnant and 14 days old pregnant WT and KO animals were studied by flow cytometry for lymphocyte subpopulation ratio and the placenta was examined by realtime PCR for placental cytokine and histamine receptor expressions.

There was no difference in the splenic lymphocyte ratios between WT and KO pregnant mice. On the other hand we found that IFN-γ expression was significantly higher in KO placentae. The expression of IL-12 was higher and the expression of IL-4 and IL-10 were lower in KO placentae than in WT, but not significantly. Interestingly the expression of H3 receptor was considerable in the placenta of both groups.

Our results suggest that during pregnancy the possible influence of histamine on the establishment of Th balance is rather local, at the site of materno-placental interface. This leads us to focus our further studies on this territory.

Toll-like receptors (TLRs) and histamine receptor H4 in articular tissues of patients with rheumatoid arthritis (RA)

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There are convincing data that histamine participates in the pathogenesis of rheumatic diseases, but the modulation of histamine production in the inflamed tissues of joints is not well known.
Since, stimulation of toll-like receptors (TLRs) and histamine receptor 4 (H4) may affect histamine production, aim of the study was to detect these receptors in the articular tissues of RA patients.
Samples of synovium and cartilage were obtained at joint replacement surgery of RA patients or from joints of amputated legs of control, age-matched patients severly injured following accidents. The receptor proteins were demonstrated by immunohistochemistry using polyclonal antibodies generated against H4, TLR2, TLR3, and TLR4 antigens.
Histamine receptor H4 was found in a major portion of chondrocytes (55%), numerous cells of pannus and synovium. A variable portion of chondrocytes and cells of pannus within each RA specimen were shown to be positive for TLR2 and TLR4. However, immunodetection of TLR3 was demonstrated in numerous cells of pannus, especially in the area of tissue erosions (synovium – cartilage junction). In synovium, TLR3 protein was found in cells of blood vessel walls and these infiltrating perivascular area. Expression of all receptor proteins appeared as intracellular immunostaining. In electron microscope TLR3 was detected in vesicular structures of cytoplasm and TLR2 was detected on the cell surface. The histamine H4 and Toll-like receptor proteins were not found in the samples of young control patients.
The results show that histamine H4 and TLRs are present in cells of RA articular tissues. Thus, they may participate in the pathogenesis of RA symptoms. However, the relation of histamine production to stimulation of all these receptors requires further studies.

Histamine and oxygen radicals of blood in the course of cardiopulmonary bypass (CPB)

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Cardiopulmonary bypass (CPB) results in development of a systemic inflammatory response syndrome (SIRS) which is believed to contribute to postoperative complication. The magnitude of the inflammation following CPB adversely influences clinical outcomes.In this study we evaluated the effect of CPB on the inflammmatory activity of the blood cells.

Blood samples were obtained from the arterial line of paediatric patients operated on for congenital heart anomalies. Samples were collected at the onset of CPB, during hypotermia and at the end of CPB. An additional samples were taken from ultrafiltration fluid (UF).The blood concentration of histamine, release of histamine from blood lymphocytes and respiratory burst of PMN leukocytes were estimated.

Results of this study showed that CPB enhances the respiratory burst of peripheral blood PMNs. The oxidative response of PMNs increased during the course of CPB and was the highest at the end of CPB, In addition, on incubation with ultrafiltrated fluid, PMNs (isolated from healthy blood donors) showed (dose- dependent) increased generation of oxygen radicals. This effect is connected with priming or activation of PMNs by inflammatory mediators present in UF.

Blood concentration of histamine was stable during CPB. In some patients we observed slight decrease in histamine concentration during hypotermia. CPB did not alter also spontaneous and stimulated (PHA) release of histamine from blood lymphocytes.

Our findings suggest that toxic oxygen radicals produced by PMN leukocytes during CPB but not histamine may participate in the development of a SIRS.

Taurine – chloramine is a potent antiinflammatory substance

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We have previously shown that taurine chloramine (Tau-Cl) a physiological compound exerting some antiinflammatory activities, modified the development of adjuvant arthritis in rats. In the present study we compared antiinflammatoty effect of Tau-Cl with effectiveness of some antiinflammatory drugs on adjuvant-induced arthritis (AA) in rats.

AA was induced in male Brown-Norway rats by injection 0.1 ml of ground heat-killed Mycobacterium tuberculosis suspended in incomplete Freund's adjuvant into the foodpad. Rats received taurine Tau-Cl (5ml 10mM i.p.) or drugs (i.p.) (hydrocortison - 5mg/kg body weight, diclofenac - 5mg/kg body weight, meloxicam 0.5 mg/kg body weight) once a day for 21 days. Treatment started simultaneously with AA. The blood concentration of histamine, release of histamine from peritoneal mast cells and respiratory burst of neutrophils were estimated.

We have confirmed that Tau-Cl decreased foodpad swelling, down-regulated elevated in AA histamine level in blood, normalized of neutrophils response to stimulation and diminished ability of peritoneal mast cells to spontaneous and 48/80 – stimulated release of histamine. Meloxicam was found to be the most effective antiinflammatory drug and its potency was comparable to Tau-Cl when the joint swelling was estimated. Less effective were hydrocortison and diclofenac. Histamine blood level was lowered by all substances tested with the potency: meloxicam> Tau-Cl=hydrocortison=diclofenac. The same degree of histamine release inhibition from mast cells was registered for all drugs. It was also true (except a low activity of hydrocortison) when the respiratory burst of neutroophils was estimated.

It is concluded that the antiinflammatory effect of Tau-Cl is as potent as an effect of commonly used in the clinic antiphlogistic drugs.

The Mechanisms of Histamine Release and IgE Synthesis Induced by Trichotecenic Mycotoxins

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Trichotecenic mycotoxins are a group of toxins produced by the Fusarium fungi found on many crops, and also by other genera such as Stachybotrys. Three of the better known toxins are T-2, HT-2 toxin and deoxynivalenol-DON (vomitoxin). The interactions between different microbial exposures have been studied by Norn (1993), who concluded that the exposure to fungal spores enhances the histamine release triggerd by both allergic and non-immunologic mechanisms in the cultured leucocytes. In present investigation is has been shown that T-2 toxin and DON dose-dependently increased histamine release from rat peritoneal mast cells activated by compound 48/80 and specific allergen from ragweed pollen. Low doses of studied mycotoxins increased the total IgE synthesis in the culture of MNC of healthy voluntaries and induced co-stimulatory effect on the specific anti-IgE-antibody synthesis. The histamine releasing effects and IgE-modulatory effects of T-2 toxin and DON, highly dependent on the functioning of different histamine receptors. Inhibition of H_1 receptors by specific antagonist loratadine in comparison to inhibition of H_2 and H3/H4 histamine receptors revealed more profound inhibitory effects on in vivo (in mice) and in vitro (MNC culture) IgE response and histamine release induced by mycotoxins.

TGF-β Modifies the Gene Expression Profile of in vitro differentiated Human Mast Cells

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TGF-β seems to be crucial in the differentiation of murine mucosal mast cells. This cytokine has also been shown to modulate the chemotaxis and FcεRI-dependent activation of human mast cells. Airway smooth muscle cells, gut epithelial cells and some tumors can produce TGF-β. As this cytokine seems to play an important role in the differentiation/function of mast cells, we analysed the TGF-β-induced gene expression changes by DNA-microarray.

Progenitors isolated from cord blood were cultured for 5-6 weeks and then TGF-βI was added. mRNA samples were linearly amplified and gene expression profiling was carried out by Agilent oligonucleotide microarray. Some results were verified by real-time PCR or flow cytometry.

More than 200 genes out of the >22,000 showed a transcriptional increase after TGF-β treatment. Some chemokine receptors (CXCR4 and CCR7), IL-3 receptor α, IL-1 receptor antagonist or the activin A (the member of the TGF-β family) receptor were upregulated. The level of leukotriene C4 synthase and prostaglandin endoperoxide synthase 1 (COX1) was also higher after TGF-treatment which shows the enhanced inflammatory mediator synthesizing capability of mast cells. On the other hand, more than 200 genes showed decreased expression, among them some proteases, e.g. cathepsin G, L, K, Z and MMP-9. The mRNA level of SCF-receptor, macrophage inhibitory cytokine 1 or IL-8 was also lower. Further interesting genes showing a decreased expression are galectin 3, heparan glucosaminyl N-sulfotransferase and chemokine ligand 13. Interestingly, histidine decarboxylase level was also lower in the treated cultures, but there was no difference in the expression of the histamine receptors.

Taken together DNA-chip results show that TGF-β can alter not only the metabolism, but also the cytokine producing, protease secreting and chemotactic properties of human mast cells.

Increased Antigen Presentation and Th1 Polarisation in Genetically Histamine-Free Mice

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Histamine, a well-known inflammatory mediator, has been implicated in various immunmodulatory effects and in the development of antigen-specific immune responses. Dendritic cells (DC) are the most potent antigen presenting cells, specialised for capture, uptake, transport, processing and presentation of antigens to T cells thus DCs play an important role in both innate and adaptive immunity against pathogens.
In this study we examined the effects of histamine on dendritic cells, especially on their antigen presenting capacity and cytokine production.
Our experiments were performed on genetically histamine-free (histidine-decarboxylase knock out (HDC KO)) mice.
According to our *in vitro* results both the HDC KO mice spleen cells and spleen derived CD11c microbead selected dendritic cells display a higher efficiency in antigen presentation compared to wild type mice.
As cytokines have important role in antigen presentation and Th-cell polarization, we focused on the expression profile of cytokines produced by dendritic cells.
We detected higher mRNA expression of Th1 cytokines in HDC KO DCs: γIFN, IL-12p35 and the most relevant Th1 transcription factor T-bet (real time PCR).
Since Th1 cytokines are known to enhance antigen presentation it can be the cause of enhanced antigen presentation of HDC KO dendritic cell.

Signal Transduction and Activator of Transcription (STAT) is Phosphoregulated by Histamine in Human Lymphocytes ex vivo – New Aspects

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Histamine is able to modulate the Th1/Th2 lymphocyte balance and allergic reactions are associated with excessive histamine production causing a shift to Th2 cell recruitment. In this study we aim to investigate whether histamine influences downstream signalling events of the histamine receptor (H) induced crosstalk and how it modulates the JAK/STAT pathway.
PBMC from 6 atopics (adult, IgE>1000 IU) as well as 6 sex and age matched non-atopics (IgE<100 IU) were stimulated 3 days with PHA, human mast cell line-1 (HMC-1) with PMA respectively. Agents were added 4 hours post-plating. The MTT-assay was used to examine cell proliferation, Western blots for determination of latent and activated(a) STAT1/STAT6. EMSA was performed for binding tests with STAT1 oligoDNA *sc-2573* and the mutant oligoDNA *sc-2574* (Santa Cruz Inc, California).
Histamine inhibited the lymphocyte proliferation, however, rIL-4 and more effectively rIL-13, induced cell proliferation. This effect was significantly enhanced in the non-atopic cells ($p<0.001$). We found that response to rIL-4 correlated with latent STAT6 expression. Anti-aSTAT6 indicated both histamine induced negative feedback, and rIL-4 to stimulate aSTAT6 activation in the atopic cells and HMC-1. STAT1 and aSTAT1 were counterparts to STAT6 and cleaved a 28kD-fragment after 3 days. Thioperamide (H3-antagonist) and clobenpropit (H4-agonist) as well as STAT1 DNA binding experiments (EMSA) indicated a crucial role for H4-receptors in signal transduction processes with STAT1 consensus and mutant oligonucleotides.
It can be suggested that histamine influences the IL-4/IL-13 induced JAK/STAT pathway predominantly in atopic and HMC-1 cells by modulating distinct histamine receptor induced signal transduction. This may explain the up-regulation of autocrine stimulated Th2 cells in atopic patients.

Histamine Stimulates Tumor Proliferation and Angiogenesis through H2/H4 Receptor-Mediated Cyclooxigenase-2 Activation in Colorectal Cancer.

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Both expression and activity of histidine decarboxylase (HDC), the key enzyme in the synthesis of histamine, have been shown to be increased in several types of human tumors. We attempted to establish whether the possible involvement of histamine in colorectal carcinogenesis might be mediated by the activation of the cyclooxygenase-2 (COX-2) pathway.
Expression of HDC was analyzed in colorectal cancer samples and corresponding normal mucosa obtained from 33 surgical specimens. We also evaluated HDC activity, histamine content and prostaglandin E2 (PGE2) production in the same specimens. The effects of histamine and H1, H2 and H4 receptor antagonists on COX-2 and VEGF expression, cell proliferation, PGE2 levels and VEGF production were assessed in three colon cancer lines (HT29, Caco-2 and HCT116) with different COX-2 expression.
Most of the tumors showed up-regulation of HDC protein expression when compared with the corresponding normal mucosa. HDC activity and histamine content were significantly higher in stage III-IV tumors than in stage I-II ones. These parameters significantly correlated with PGE2 production. The administration of histamine increased COX-2 and VEGF protein expression, cell proliferation, PGE2 levels and VEGF production in the COX-2 positive HT29 and Caco-2 cells. Treatment with either H2/H4 receptor antagonists or celecoxib could reverse these histamine-mediated effects. Treatment of the COX-2 negative HCT116 cells with histamine had no effect on both the COX-2 pathway and VEGF expression/production.
Our data showed that up-regulation of HDC activity was correlated with metastatic disease in colorectal cancer. We found that histamine might exert both a pro-proliferative and a pro-angiogenic effect through the activation of the H2 and H4 receptors. It is likely that these effects are mainly mediated by increasing COX-2-related PGE2 production in COX-2 expressing colon cancer cells.

Comparison of the Differentiation Capacity of Histamine Deficient(HDC KO) and Wild Type Embryonic Stem (ES) Cells

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Although the major function of histamine in allergic reactions is known for decades, and its involvement in other cellular processes e.g. in cell proliferation has been described recently, there is a few known about its role in embryonic cell differentiation. By comparing the differentiation capacities of histamine deficient, HDC -/- and wild type ES cells we tried to elucidate the role of histamine in the cellular differentiation.

Using the protocol developed by A. Wobus, ES cells differentiate efficiently into functional cardiac cells. In our experiment at different timepoints number of spontaneously beating clusters in embryoid bodies (EB-s) and beating frequency of cardiomyocytes were determined. At the same time points nestin, titin, desmin and the ES specific Oct4 and SSEA-1 expression were checked by immunocytochemistry. Expression of all four histamine receptors was also checked by RT-PCR or by real-time PCR both in differentiated and undifferentiated states. No H3 and H4 histamine receptor expressions were detected, while both H1 and H2 receptors were expressed in both control and KO cells before and after differentiation. Based on the results of experiments, it seems that the HDC -/- ES cells produce smaller EB-s, their differentiation to cardiomyocytes takes a longer time. On the other hand contraction frequencies of both HDC KO and WT ES derived cardiomyocytes were reduced by the addition 10^{-5} M histamine.

As for all checked immunocytochemical markers HDC -/- ES showed a less intensive expression than their wild type counterparts. By adding histamine these expression patterns were modified. It seems that histamine not only acts on beating frequency of cardiomyocytes at the time of anaphylaxis but it can also influence the cardiomyogenesis.

Is there any influence of the absence of H4 receptor on T cell development: comparison of T cell precursors and thymic stroma cells involved in thymic selection of H4 receptor knockout (H4R-KO) and wild type (WT) mice

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Background: Hemopoiesis is regulated by different factors including hemopoietic growth factors, chemokines, cytokines and different low molecular weight substances, like histamine. The effect of histamine, however, is markedly dependent on the types of histamine receptors expressed on target cells. Hemopoietic cells express H1-, H2 and H4 receptors on their surface.

Aim: To study the T cell precursors in thymus of H4R-knockout and wild type mice and to characterize of thymic cells involved in positive and negative selections. The expression of costimulatory (CD28, CD80, CD86 and CTLA4) and regulatory (IL3Rα and β, IL7Rα, glucocorticoid receptor=GCR) molecules were also compared between the groups. All measurements were performed by flow cytometry.

Methods: Multicolor flow cytometric stainings were used for all measurements. Fluorescence intensity values were used for comparison of costimulatory and regulatory molecules expressions.

Results: Significantly higher percentages of pro T cells and triple positive late pre T cells were found in the thymus in H4R-KO mice, but there were no differences in the mature T cell subsets. Apoptosis plays a critical role in the development of T cell repertoire. In H4R-KO mice the percentage of thymic CD95L+ cells was elevated, while the percentage of CD11c^{high}+ dendritic cells decreased significantly in H4R free animals. Expression of CD80 and CD86 co-stimulatory molecules on the surface of thymic cells was lower in H4R-KO mice, but this difference proved to be significant only in the case of CD80.No any differences were found in the percentage and the expression of IL7Rα positive thymic or bone marrow cells and in the amounts of IL3Rβ expression of bone marrow cells while the percentage of IL3Rα+/CD25+ cells in bone marrow increased in H4R-KO mice. CD25 negative thymocytes showed lower GCR expression compared to CD25 positive thymocytes in both groups while there no any differences were detected between H4R-KO and wild type mice.

Conclusion: Our results suggest that the lack of the effect of histamine through H4 receptor has a general impact on T cell differentiation. Whether the detected differences were caused by the direct effect of histamine on T cells or on the thymic stroma cells involved in thymic selection, remains to be elucidated.

Pharmacokinetic Effects of a Single Dose of Antidepressant on Histamine in the Rat

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Plentiful substances are known that affect histamine kinetics yet relatively little data have been available about effects of antidepressants. Our previous studies have shown that antidepressants, either tricyclic amitriptyline (AM) or selective 5-HT uptake inhibitor citalopram (CIT) given short period before intravenous bolus dose of histamine inhibit increase of the amine concentration in plasma. Moreover, they also inhibit histamine induced cardiovascular effects in the rat. In the present work we were interested in long-term effects of a single dose of AM or CIT on plasma histamine in the rat. We compared them with effects of 5-HT uptake inhibitors fluvoxamine (FLU) and sertraline (SER) as well as atypical antidepressant mianserin (MIA) on compound 48/80 induced changes in endogenous plasma histamine levels. AM or CIT were given intraperitoneally 24 hours before intravenous injection of histamine liberator, compound 48/80 or exogenous histamine (iv, 10 µg/kg) while FLU, SER or MIA were given 15 min before c. 48/80 (0.05 µg/g). Plasma histamine levels were followed and showed that FLU, SER and MIA diminish the increase of the amine levels caused by c. 48/80 and increased the rate of histamine disappearance from plasma. The effects of AM and CIT on endogenous histamine were comparable to those of other antidepressants in the study: inhibition of c. 48/80 induced increase in plasma histamine levels and faster elimination of the amine from plasma. Effects on histamine changes caused by exogenous histamine administered 24 hours after AM- and CIT-treatment were also significant showing inhibitory effect on the increase in plasma histamine concentrations. It can be concluded that antidepressants – regardless of the type, show very fast and long lasting significant effect on pharmacokinetics of histamine. Could this result be regarded as a useful “side effect”?

Origins of plasma amine oxidases in different mammalian species

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The blood plasma of mammals contains considerable activity of soluble copper-containing amine oxidases (AOCs) referred to as plasma amine oxidases. The identity and tissue origin of plasma amine oxidase has been a mystery until AOC genes and proteins were characterized. Mammals possess up to four AOC genes. AOC1 encodes diamine oxidase (DAO), the enzyme inactivating histamine and other diamines. DAO is a soluble protein expressed preferentially in kidney and intestine. DAO is stored in plasma membrane associated vesicles in DAO producing cells and is secreted upon stimulation. AOC2 encodes retina amine oxidase (RAO), presumably a plasma membrane protein originally identified in the ganglion cell layer of the retina. Neither the enzymatic properties nor the function of this enzyme have been studied. AOC3 encodes vascular adhesion protein-1 (VAP-1), a peripheral plasma membrane protein with monoamine oxidase activity. VAP-1 is expressed in vascular endothelial and smooth muscle cells and has been implicated in leukocyte extravasation. Recent experiments revealed that the large extracellular domain of VAP-1 can be shed from the cell surface by a protease to give rise to soluble VAP-1 present in blood plasma. Additionally, an AOC4 gene was characterized whose gene product is highly homologous to VAP-1 and has a signal peptide rather than a transmembrane domain at its N-terminus. AOC4 is expressed primarily in the liver from where it is secreted into the bloodstream to constitute the major part of plasma amine oxidase activity in most mammalian species. Interestingly, the human genome contains a complete AOC4 gene whose reading frame is interrupted by an internal stop codon whereas the genomes of mouse and rat contain only fragments of an AOC4 gene. Both humans and rodents have comparably low plasma amine oxidase activity that is probably derived only from soluble VAP-1.

New Structural Insights to Search for Selective Mammalian Histidine Decarboxylase Inhibitors

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Mammalian histidine decarboxylase (HDC) is a PLP-dependent enzyme highly relevant to inflammatory diseases. At present, all inhibitors developped against the mammalian enzyme are also active against the one of enterobacteria, blocking the possibility to avoid several secondary effects when orally administered. We have built a first 3D model for active mammalian HDC by comparative modeling techniques (Rodríguez-Caso *et al.*, 2003, Eur. J. Biochem., 270: 4376-87). The predicted model has been validated by results from more than 30 different mutants (Fleming *et al.*, 2004, Biochem. J., 379: 253-61; and unpublished results). New information arise from its analysis about potential target of selective intervention. One of them is the catalytic site surroundings, located between both monomers. Most of the residues involved in PLP-binding are located in one of the monomers; however, both monomers contribute to substrate entrance and binding, which provides new possibilities to modify substrate availability and catalytic efficiency by interfering dimerization surface (Moya-García *et al.*, 2005, BioEssays, 27: 57-63. A biocomputational analysis of the dimerization surface is done looking for specificity determinants in the PLP-dependent decarboxylase evolution, so that new potential targets for selective inhibition are detected. Among others, this study point out an ionic patch present in the dimerization surface close to the catalytic site. Experimental data from direct-mutant versions strongly support our hipothesis. Our results therefore indicate that looking for a high affinity ligand to this ionic patch could provide a selective way to inhibit mammalian HDC, but not the bacterial PLP-dependent activity. It clearly open a new way to search for new drugs interfering with histamine synthesis in mammalian tisúes. Granted by SAF2002-2586 (MEC), REMA (FIS) and GNV-5 (INB).

Antiradical Effects of Antihistamines. Structure-Activity Relationship.

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Previously we found that the H_1-histamine receptor antagonist dithiaden decreased formation of reactive oxygen species and that in this effect also non-receptor interference with neutrophil membranes could be involved [1,2]. This led to the assumption that halogen substitution in the antihistamine molecule, increasing affinity of drugs to biological membranes, may potentiate antiradical activity. To confirm this, effects of pheniramine, chlorpheniramine and brompheniramine were compared. Concentration of oxygen metabolites in whole human blood was measured by the luminol-enhanced chemiluminescence method in a microtitre plate computer-driven luminometer. Physico-chemical parameters were calculated for optimum structures of the drugs tested, using appropriate computer programmes. Brompheniramine proved to be the most effective. This drug, in 10 μmol/l concentration, decreased chemiluminescence stimulated with PMA (phorbol-12-myristate-13-acetate), opsonised zymosan and Ca^{2+}-ionophore A23187 by 38%, 25% and 22%, and in the 10-times higher concentration it induced 97%, 81% and 50% inhibition, respectively. The effect of chlorpheniramine (100 μmol/l) was less pronounced – reduction of chemiluminescence by 61% (PMA), 40% (zymosan) and 21% (A23187). In the presence of pheniramine maximum inhibition did not exceed 15%. The calculated partition coefficients, indices of molar refraction and dipole moments of the drugs tested declined in the following order: brompheniramine > chlorpheniramine > pheniramine, i.e. parallelly to the inhibition of chemiluminescence. Halogenation of pheniramine potentiated its antiradical activity, indicating that increased affinity to membranes might be involved in this effect.

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H_1-Antihistamines and Activated Blood Platelets

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H_1-antihistamines possess side effects which might involve beneficial therapeutic activities. Depending on their physicochemical properties, they showed antiinflammatory effects, decreased platelet aggregation and suppressed phagocyte activation. We compared the in vitro effect of H_1-antihistamines dithiaden (DIT) and bromadryl (BRO), with the terminal methyl or ethyl groups in the side chain, with loratadine (LOR), a nonsedating polycyclic histamine H_1 antagonist, on aggregation of human blood platelets stimulated with receptor operated (ADP) and receptor bypassing (PMA) stimuli. Blood was taken from healthy volunteers and anticoagulated with 3.8 % sodium citrate (ratio 1:9). Platelet aggregation was measured in whole human blood (impedance aggregometry) and turbidimetrically in platelet rich plasma (PRP) and in isolated platelets. BRO, DIT and LOR alternatively decreased platelet aggregation in a dose-dependent manner. In whole blood aggregation induced with ADP or PMA, 20 and 50 μmol/l concentrations of DIT and BRO proved effective, whereas LOR was ineffective in these concentrations. The aggregation of platelets in plasma induced with ADP was significantly inhibited by DIT (10 μmol/l), BRO (100 μmol/l) and not affected by LOR. In isolated platelets all drugs tested inhibited PMA-stimulated aggregation in the following rank order: BRO>DIT≥LOR. The representatives of the 1^{st} generation of H_1-antihistamines were much more effective in their antiplatelet activities as compared with LOR. The absence of antiplatelet effect of LOR in whole blood and PRP might result from its high affinity to plasma proteins. Interaction with aggregation stimulated with PMA in isolated platelets resulted most probably from inhibition due to LOR at intracellular level. The antiaggregatory activity of BRO and DIT indicated interaction at receptor stimulatory pathway (ADP) as well as intracellularly (PMA).

Extra- and Intracellular Oxidant Production in Phorbol Myristate Acetate Stimulated Human Polymorphonuclear Leukocytes: Modulation by Histamine and H_1- Antagonist Loratadine®

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The generation of reactive oxygen metabolites (ROM) in polymorphonuclear leukocytes (PMNL) might be modulated by mediators released from surrounding tissues during inflammation and allergic responses, as well as pharmacolgically by drugs which are useful in the therapy of these processes.
We evaluated the effect of histamine (HIS) and the H_1-antagonist Loratadine® (LOR) on ROM generation in PMNL stimulated with phorbol-12-myristate-13-acetate (PMA) in relation to tissue destruction (outside of PMNL) and to microbicidal events (inside PMNL).
Isolated PMNL were exposed to HIS or LOR (0.1-100 μmol/L) and subsequently stimulated with PMA (0.05 μmol/L). The production of ROM was investigated using luminol or isoluminol enhanced chemiluminescence (CL). The extracellular CL was estimated in the system containing isoluminol and horseradish peroxidase as a limiting factor for CL intensity. The intracellular CL was measured with luminol in the presence of the extracellular scavengers superoxide dismutase and catalase. The CL signals were measured in microplate luminometer Immunotech LM-01T (Immunotech, Czech Republic).
After stimulation of PMNL with PMA, both extra - and intracellular ROM generation was detected. HIS in the concentrations of 1,10, and 100 μmol/L significantly increased extracellular CL, but did not change the intracellular CL in PMA stimulated PMA. LOR in the concentrations of 10 and 100 μmol/L significantly decreased both extra- and intracellular CL.
The obtained results suggest that in human PMNL stimulated by PMA, HIS might contribute to tissue destruction, as it potentiated extracellular CL. LOR depressed extracellular ROM generation and thus may decrease the extent of PMNL-mediated tissue injury. The LOR induced inhibition of intracellular CL can however be expected to diminish the physiological response of PMNL.
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Protective Effect of Clemastine against Compound 48/80-Induced Drop of Mean Arterial Blood Pressure in the Rat

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Release of histamine and other inflammatory mediators from serosal mast cells triggered by compound 48/80 is manifested as a sudden drop of blood pressure *in vivo*. In the present study a protective effect of H1-antagonist clemastine against compound 48/80-induced drop of mean arterial blood pressure (MAP) was investigated.
MAP was recorded in the abdominal aorta of anesthetized male Wistar rats (370-500 g, four rats per group) with Millar SPR-407 pressure catheter. Five min after the start MAP recording, saline (2.5 ml/kg; 1 ml/15 min) or clemastine (2.5 ml/kg; 2 mg/kg; 1ml /15 min) were infused into the left femoral vein. Following infusion, at t = 20 min, compound 48/80 was administered as intravenous bolus injection (0.3 mg/kg) into the left femoral vein. MAP was continuously recorded until t = 60 min.
In the saline pre-treated group, after compound 48/80 administration, MAP dropped from initial 72.7 ± 3.9 mm Hg to 36.6 ± 3.4 mm Hg in a period of 7 min. After seven min a progressive increase of MAP was detected. However, a complete recovery of MAP to its initial values was not observed. In the clemastine pre-treated group compound 48/80 evoked a drop of MAP from initial 73.2 ± 3.6 mm Hg to 51.2 ± 2.7 mm Hg in a period of 4 min. After four min a progressive increase of MAP was detected and a complete recovery of MAP up to its initial values was observed. Protective effect of clemastine on compound 48/80-induced drop of MAP was statistically evaluated by two-way repeated measures ANOVA and a significant protective effect of clemastine was determined ($p = 0.006$).
In conclusion, clemastine produced only a partial protection against compound 48/80-induced drop of MAP. Therefore, other mediators released from mast cells are probably also responsible for the reduction of MAP evoked by compound 48/80.

Regulation of Nerve Growth Factor-Induced Histamine and Arachidonic Acid Release from Rat Mast Cells by Cannabinoids

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Nerve growth factor (NGF) is an endogenous peptide that in addition to neurotrophic activity regulates immune system, which is critically important in the development of inflammation. The activation of mast cells by NGF evokes release of several inflammatory mediators, such as histamine, arachidonic acid and different cytokines. *In vivo* studies have reported that endogenous cannabinoids accumulate in inflammatory tissues, where down-modulate mast cell activation and reduce tissue inflammation. In our present study we investigated whether histamine and arachidonic acid release induced by NGF could be suppressed by pre-treatment of rat mast cells with cannabinoids. As cannabimimetic compounds we used different cannabinoid (CB) agonists (R-(+)-methanadamide (MA), JWH 015, palmitoylethanolamide (PEA)) and antagonists (AM 251, AM 630).

Our results show that pre-treatment of rat mast cells by cannabinoid agonists do not suppress neither histamine nor arachidonic acid release induced by NGF. Furthermore, JWH 015, a selective CB2 agonist, slightly enhances histamine release induced by NGF. Treatment of mast cells with PMSF, a fatty acid amide hydrolase inhibitor, was without effect on the release histamine. Therefore, the lack of effect of cannabinoids could not be due to the degradation of cannabinoids by fatty acid amide hydrolase. In addition, our results indicate that release of arachidonic acid induced by NGF was enhanced when mast cells were preincubated with MA, a selective CB1 agonist. The potentiating effect of MA on NGF-induced arachidonic acid release was not significantly affected by AM 251, a selective CB1 antagonist. These results suggest a non-specific effect of cannabinoids on mast cells. We can conclude that anti-inflammatory action of cannabinoids is not the consequence of the reduced release of histamine and arachidonic acid from rat mast cells.

Evaluation of the Effects of a Novel Carbon Monoxide Releasing Molecule (CORM-3) In an *In Vitro* Model of Cardiovascular Inflammation.

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The heme oxygenase (HO) is a family of enzymes able to split the tetrapyrrole heme ring to biliverdin, free ferrous iron, and carbon monoxide (CO). At least two isoforms of heme oxygenase are expressed in mammalian cells, HO-1 the inducible isoform and HO-2, the constitutive one. In particular, HO-1 is a stress-responsive enzyme that acts during inflammatory reactions, regulating immunological responses involved in cardiac anaphylaxis, in allergic reactions and in the rejection of transplanted organs. Previous reports from our group showed that exogenous CO or water-unsoluble CO-releasing molecules were able to mimic the anti-allergic and anti-anaphylactic effects of HO-1 in isolated guinea pig hearts, in guinea pig mast cells and in human basophils, mainly through the activation of the soluble guanylyl cyclase. Here we report the effects of a novel, water soluble CO-releasing molecule (CORM-3) in an *in vitro* model of cardiovascular inflammation. The anti-inflammatory properties of CORM-3 were evaluated in a coincubation of rat coronary endothelial cells (ECs) with human neutrophils (PMN), activated with the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP, 10^{-8}M), through the flow cytomeytric evaluation of cellular surface markers (CD54 expressed from endothelial cells and CD11b expressed from neutrophils).
The expression of CD54 upon EC membrane was increased after the incubation with PMN stimulated by fMLP. CORM-3 (100nM-10uM) was able to reduce the activation of EC, while the inactivated form of the drug (iCORM), unable to release CO, was ineffective. PMN significantly increased the production of ROS upon activation with fMLP and, consistently with the hypothesis that superoxide anion plays a role in endothelium activation, the treatment of the cells with SOD (300 IU/ml) mimicked the effects of CORM-3. Finally, CORM-3 also reduced the activation of human PMN, assessed as the membrane expression of CD11b. The inactivated form of CORM-3 and SOD were ineffective. CORM-3 was also able to induce the production of cGMP in the treated samples.
In conclusion, CORM-3 was highly effective in the reduction of PMN-induced CD54 expression upon EC. The effect was mediated by the release of CO, since the inactivated form of CORM-3 was completely ineffective. We can also suggest an involvement of superoxide anion, since the activation of EC was reverted incubating the cells with SOD and an involvement of a cGMP dependent intracellular pathway.

The Effect of H_1-Antihistamines on Oxidative Burst of Phagocytes

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As observed in our previous study (1), H_1-antagonist dithiaden inhibits the production of reactive oxygen metabolites by phagocytes, which is the essential defensive mechanism against microbial pathogens. The aim of the present study was to compare the inhibition effect of dithiaden with effects of four selected H_1-antihistamines of the 2nd generation - loratadine, acrivastine, astemizole and ketotifen fumarate.
Rat polymorphonuclear leukocytes were isolated from peripheral blood by dextran sedimentation. Phagocytes were stimulated with opsonized zymosan in the presence/absence of antihistamines in the concentration range of 0.001 – 0.5 mmol/l. The production of reactive oxygen metabolites by phagocytes was analysed using microtitre plate luminometer Immunotech LM-01T, chemiluminescence (CL) activity was monitored for 60 min.
Ketotifen fumarate inhibited CL activity of phagocytes in the concentrations higher than 0.01 mmol/l. This effect was similar to the effect of dithiaden. Astemizole was even more effective when only 0.001 mmol/l concentration did not affect the CL of phagocytes. In contrary, loratadine inhibited CL of phagocytes in the highest concentration only and no inhibition effects of acrivastine were observed.
Presented data showed that acrivastine and loratadine do not suppress the key microbicidal system of phagocytes. That is why these antihistamines should be used preferentially when possible.
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Histamine and PGE_2 Stimulate the Production of Interleukins-6 and -8 by Human Articular Chondrocytes in vitro.

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Background: Histamine is an important modulator of numerous physiological processes and modifies the behaviour of all cell types expressing histamine (H) receptors. It is known to modulate the production of cytokines and their receptors. Human articular chondrocytes (HAC) express both H_1 and H_2 receptors[1] and have been shown to express histidine decarboxylase (HDC) and H in human osteoarthritic cartilage in situ[2].

Objectives: To demonstrate the cytokine response of HAC in vitro following H exposure, especially the induction of interleukins(IL)-6 and -8.

Methods: HAC cultures (n = 8, all passage 2 or 3) were treated with and without histamine (20μM)and the conditioned culture medium after 24 or 48h was assayed for cytokines using Beadlyte and ELISA technology; results are presented relative to 10^6 cells.

Results: Beadlyte analysis of ten cytokines showed that HAC IL-6 and -8 were produced in far greater quantities than others, both being stimulated by H exposure. This induction was inhibited by mepyramine indicating an H_1 receptor pathway, but not by ranitidine, an H_2R antagonist. However, since this H_1R pathway also increases prostaglandin synthesis, the effect of PGE_2 on IL-6 and IL-8 expression was examined. PGE_2 alone stimulated the production of both cytokines, to a greater extent than H alone. Whereas H induced an approx. 50% increase in cytokine production, PGE_2-induction was significantly greater. Indeed, indomethacin prevented the H-induced increase in the IL-6 and IL-8 production, showing that PGE_2 was the primary stimulus for the cytokine induction.

Conclusions: Both IL-6 and IL-8 production by HAC in vitro were stimulated by H exposure, mediated via the H_1 receptor pathway. However, this induction was primarily mediated via PGE_2 expression rather than by a direct action of H on the HAC.

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Bone Marrow Regeneration and IL-3 Expression are Delayed in Histidine Decarboxylase Knock Out (HDC-ko) Mice

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Earlier a difference was detected in the percentages of immunophenotypically characterised bone marrow (BM) cell populations between HDC-ko and wild type (WT) mice. In the following study the effect of 4 Gy whole body irradiation on BM cell populations and IL-3 receptor expression was compared in WT and HDC-ko mice.

HDC-ko and WT mice were subjected to a total dose of 4 Gy whole body gamma-irradiation. Bone marrow samples were obtained on the 1st, 3rd and 7th days. Using flow cytometry, regenerating cell populations were immunophenotypically characterised: Lin- population, CD34+ cells, ckit+ cells, long-term (CD34-/ckit+/Sca1+/Lin-) and short-term (CD34+/ckit+/Sca1+/Lin-) repopulating cells were distinguished. Also, the surface expression of IL-3R α and β chains on these cell populations was determined. In WT mice intracellular HDC and histamine content in the BM cells was measured. Serum level of IL-3 protein was measured using ELISA.

The results show that the irradiation-induced decrease in the defined cell populations is to a greater extent in HDC-ko mice. While WT bone marrow is almost recovered by day 7, HDC-ko regeneration is delayed. Also, both HDC and histamine content of BM cells of WT mice are increased during regeneration compared to control animals. Moreover, the increase of IL-3 receptor expression on regenerating BM cells lags behind in HDC-ko mice. In accordance with BM results, the serum level of IL-3 protein is also decreased in HDC-ko mice.

It can be concluded that irradiation-induced BM depression is greater and BM regeneration is delayed in the absence of histamine. Moreover, IL-3 receptor expression on regenerating BM cells is reduced, as well as serum IL-3 concentration. These findings support the role of histamine in the regulation of hematopoiesis and BM regeneration processes.

Histamine Modulates Cellular Events Involved in Tumor Invasiveness in Pancreatic Carcinoma Cells.

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Progression of metastatic epithelial tumors is characterized by an increased motility, a decrease in cellular adhesion, high expression and activation of metalloproteases (MMP) and high angiogenesis capability. In the human pancreatic carcinoma cell line PANC-1, histamine (HA) acts as a growth factor modulating cellular proliferation via H1 and H2 histamine receptors.
The objective of this work was to study HA action on cellular adhesion and migration and the expression of MMPs in this cell line. Methylene blue staining of formaline fixed cells, between 30 and 60 minutes after seeding, was performed for studying cellular adhesion. Cells had been previously treated with HA for 48 hours. A decrease in the adhesion to plastic was seen at any tested time. At 90 minutes: Control 100%, 10 μM HA = 63%, 10 nM HA = 44%, ANOVA $p<0.01$. The scratch wound healing technique was used to evaluate cellular migration. PCNA expression was determined by flow cytometry, in order to discard cellular division. It was observed an increased motility in 10 nM HA treated cultures after 24 hours, whereas there was no difference in PCNA expression between treated and control. MMP-2 and MMP-9 activities were determined by zymography. The intensity of gelatinolytic bands was quantified by an appropriate software and results were expressed as a percentage of control values. It was determined an increase in the active form of MMP-2 in the supernatants of HA 10nM treated cultures (130% vs control 100%, Student's test $p< 0.05$).

Present data indicate that HA could be involved in tumoral progression towards metastasis in PANC-1 cell line.

Histamine influences on the Migration, Expression of Adhesion Molecules and MMPs in Human Melanoma Cell Line

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The incidence of malignant melanoma significantly increases recently. The patients with the disease exhibit reduced life expectancy. Histamine is an important paracrine and autocrine regulator of normal and tumor cell proliferation, as well. Melanoma cells reveal autonomous histamine metabolism.
Our aims were to demonstrate that histamine plays a key role in the metastatic potential of human melanoma cells. We compared the effects of histamine on the migration, expression of adhesion molecules and matrix metalloproteinases (MMP) in two human melanoma cell lines with different invasiveness.
Modified Boyden-chamber technique was applied to evaluate the effects of histamine on the migration. Immunocytochemistry and flow cytometry were carried out to evaluate the expression of adhesion molecules following histamine treatments with different concentration and administration time. Immunocytochemistry, flow cytometry and real-time RT-PCR were carried out to estimate the expression of MMP-2 and MMP-3.
We found that histamine had chemorepellent effect on both melanoma cell lines. Expression of CD44, NCAM and ICAM-1 was downregulated, while MMP-2 and MMP-3 were upregulated upon the histamine treatments. Histamine plays a key role in several steps of the metastatic cascade such as migration, adhesion and invasion.
Our *in vitro* results suggest that histamine decreases directly the migration ability of human melanoma cells. On the other hand, histamine indirectly increases the invasive potential of melanoma cells by upregulating MMP-2 and MMP-3, and by downregulating adhesion molecules.

The Role of STAT1 and 6 in Histamine-Mediated Growth Inhibition of Human Breast Carcinoma Cell Lines.

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It has been established that histamine (HA) regulates the proliferation of different human neoplastic cell lines.(1) Since aberrant activation of STAT-signaling may produce cell transformation and oncogenesis, (2) the aim of our study was to investigate the induction and activation of STAT1 and STAT6, which can be involved in growth inhibition and apoptosis of tumoral cells, after HA exposure of human breast cancer cell lines.
MCF-7 (mammary carcinoma, estrogen receptor (ER)-positive) and MDA-MB-231 (mammary carcinoma, ER-negative) cells were treated with HA (10 nM - 10 μM) for different incubation times. Cell growth was evaluated by clonogenic assay, latent and phosphorylated STAT1 and 6 were determined by western blot and STAT1 DNA binding was further investigated by electrophoretic mobility shift assay (EMSA). Apoptosis was assessed by DiOC6 staining methods and DNA fragmentation. IL-4 and INFγ production was assayed by ELISA.
Our results show the ability of HA to modulate the activation of STAT1 and 6.
HA 10 μM treatment suppressed the proliferation of both malignant cell lines. Accordingly, HA not only increased latent STAT1α isoform and its activity but also stimulated STAT6 in MDA-MB-231 cells. Lower HA concentrations showed an increase in cell growth, but did not exert any effect on STAT activation. In contrast, HA decreased the latent STAT1β isoform and the STAT1 DNA binding while augmenting activated STAT6 in MFC-7 cells.
Cytokine production was nearly undetectable and it was not modified by HA treatment.
This study indicates that STAT signaling may be an important mediator of HA action in breast cancer cells.

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Histamine Stimulates Differentiation of Human Trophoblast through H1 Receptor.

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During placental implantation, trophoblast differentiate into villous and extravillous line. Extravillous trophoblast infiltrate spiral arteries and modify their epithelium what is associated with change in surface proteins. The cells of invasive trophoblast become alpha6-beta4 integrin negative and alpha5-beta1, alpha1-beta1 and alphaV-beta3 integrins positive. Poor invasion reported in preeclampsia is associated with decreased expression of the alphaV-beta3 integrin. Our previous study showed enhancement of alphaV-beta3 integrin expression in trophoblast cell culture treated with histamine.
The aim of this study was to examine the way which histamine influences on alphaV-beta3 integrin expression through in trophoblast cultured in vitro.
11 placentas were obtained after term deliveries. Trophoblast cell cultures were established using modified Kliman's method. Histamine alone or with: pyrilamine, cimetidine, clobenpropide or NaCl (0,9%) were added daily into the vessels (culture H, P, C, CB and control, respectively). After 48, 72 and 96 hours, cells were detached from vessels and expression of integrin alphaV-beta3 was measured with ELISA.
Statistical analysis of the results was performed using U-Mann-Whitney test.
The expression of integrin alphaV-beta3 examined after 48 hours differed from control group at: H 8.4%± 3.15, P -2.43%±6.87, C 6.58% ±14.59, CB 11.59% ± 13.59; $p>0.05$. Adhesion measured after 72 hours of culture differed from control group at: H 37%± 12.3, P 13.9%±6.9, C 51% ±10.5, CB 24.3% ± 13.7, $p<0.05$. Adhesion measured after 96 hours of culture differed from control group at: H 57.7%± 10.7, P -5.31%±6.29, C 43.2% ±8.4, CB 51% ± 5.6, $p<0.05$, in group P $p>0.05$.
Histamine stimulates alphaV-beta3 expression in trophoblast cells culture from term placentas after uncomplicated pregnancies acting through H1 receptor.

The Role of Histamine and Its Receptors in the Development of Ovarian Follicles *In Vitro*.

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Histamine (HA) may influence processes of follicular development and ovulation. Changes in total number and distribution of ovarian mast cells, potential sources of HA, were reported in the course of estrus cycle. We examined histamine H1,H2 and H3 receptors expression, effects of HA administration and histaminergic blockade during the development of ovarian follicles. An in vitro model with cultured murine follicles was developed. The follicles (*N*=320) were isolated from immature mice using method described by Nayudu and Osborn [1]. Selected follicles of 150-180 μm were individually cultured in culture plate inserts in medium containing 5% immature mouse serum, supplemented with 100 mIU/ml recombinant human FSH. Diameter of the follicles was measured daily using x100 magnification and a calibrated micrometer. At the end of culture period (5-6 days), follicles were induced to ovulate with hCG. After immunostaining, follicular expression of H1,H2 and H3 receptors in subsequent days of culture was investigated using morphometry for quantitative analysis. The effects of HA (10 mmol/l) and H1,H2 and H3 blockade (mepyramine, cimetidine and thioperamide, respectively) were also evaluated. In the preovulatory period we found a positive correlation between H1 and H2 expressions and the mean follicular size. Follicles matured for ovulation demonstrated the highest H1 and H2 expressions, but hCG-induced ovulation occurred almost exclusively (96,5 %) when follicular H1:H2 proportion amounted 4:1 or higher. Administration of HA increased significantly ovulation rates, while treatment with both mepyramine and cimetidine, but not thioperamide reduced incidence of hCG-induced ovulation ($p<0,05$). In conclusion, interactions between HA and H1 and H2 receptors play important role in follicular development and ovulation in vitro.

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Histamine H4 receptor on mammary epithelial cells of the human breast with different types of carcinoma

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The potential role of histamine in normal and tumour cell growth has been a subject of numerous reports. However, findings are often contradictory. The effect of exogenous histamine on tumour cell growth (in vitro) appears to vary in relation to the type of tumour cell and expression of histamine receptors on the target cells. In vivo, the effect of histamine on the tumour growth is likely to be even more complex because the expression of histamine receptors may be regulated by microenvironmental factors such as cytokine network. The purpose of the study was to evaluate expression of recently discovered histamine H4 receptor and to determine the presence and the phenotype of mast cells (as important source of histamine) in different types of human breast carcinoma.
Immunohistochemical staining with specific antibodies was used to evaluate the expression of histamine H4 receptor protein and phenotype of mast cells in the samples of invasive and non invasive human breast carcinomas and in normal mammary gland of the same patients (to avoid personal differences in the protein expression).
In many types of tumours, mast cells were often preferentially accumulated at the tumour periphery and the tryptase phenotype (MC_T) predominated (95%) over the tryptase-chymase phenotype (MC_{TC}). Different numbers of these cells showed positive staining for histamine H4 receptor depending on the section and the cancer type. Normal mammary epithelial cells express intense staining for H4 receptor, whereas carcinoma cells showed positive staining only in some benign tumour types.
It is the first report concerning the presence of histamine H4 receptor protein in the mammary epithelium of the human breast. The results suggest that through this receptor histamine may control a physiological function (eg. proliferation) of these cells and that the loss of such control may play a role in neoplasia.

Molecular cloning of guinea pig diamine oxidase and histamine N-methyltransferase

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The guinea pig has been an important model for studying histamine function in vitro and in vivo. However, the enzymes responsible for the inactivation of histamine, diamine oxidase (DAO) and histamine N-methyltransferase (HNMT), have not been characterised at the molecular level for this organism. Therefore, we cloned and analysed cDNAs encoding guinea pig DAO and HNMT to learn more about the structure and function of these proteins. Total RNA was prepared from various guinea pig tissues and reverse transcribed into cDNA. Using primers derived from other known DAO and HNMT sequences partial guinea pig DAO and HNMT cDNAs were amplified by PCR. Full-length cDNA sequences were obtained by rapid amplification of cDNA ends. Sequence analyses revealed that guinea pig DAO is a protein of 750 amino acid residues that is highly homologous to other mammalian DAO proteins. Guinea pig DAO has an N-terminal signal peptide sequence, three conserved N-glycosylation sites and possesses all structural motifs identified in other copper amine oxidases, including a conserved tyrosine that is converted to the active-site cofactor 2,4,5-trihydroxyphenylalanine quinone, three histidines that bind the copper ion, and an aspartic acid residue that functions as a catalytic base. Guinea pig HNMT has 292 amino acid residues and is highly homologous to human, pig, mouse, and rat HNMT. Amino acid residues that have been shown to interact with histamine and with the S-adenosylmethionine in human HNMT are absolutely conserved. Evolutionary the histamine inactivating enzymes from guinea pig are closer related to the pig and human proteins than to the mouse and rat proteins.

Experimental ulcerative colitis in rats; the effect of diamine oxidase administration*

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Positive effects of the application of histaminase in acute allergic asthma-like reactions in actively sensitized guinea pig have recently been reported [1]. Since ulcerative colitis (UC) is, among other pathological changes, characterized by an enhanced release of intestinal mast cell mediators and significantly reduced histaminase (diamine oxidase, DAO) activity, it was of interest to examine the effect of repeated enzyme administration in a rat model of UC. Experiments were performed on male Wistar rats (260-300 g), in accordance with EC directives and local ethical regulations. Rats were randomly allocated to 3 groups (n=8 each): control, UC, UC-treated. UC was induced by intrarectal administration of 4% (v/v) acetic acid for 15 s. Hog kidney diamine oxidase, immobilised on Concanavalin A-Sepharose was given to rats as a suspension of 25 miliunits/daily, i.p., until day 5th when animals were sacrificed. The severity of colonic lesions was evaluated by scoring, inflammation by measurements of plasma ceruloplasmin activity and myeloperoxidase activity in the colon. The tissue histamine concentration, DAO and histamine methylating enzyme activities were estimated. In UC-treated group macroscopic changes in colon were milder, the inflammatory markers lower. In contrast, diamine oxidase activity was highest in the colon of UC-treated rats, indicating that the tissue could bind the enzyme. The study supports the previous observation on the beneficial effects of the DAO preparation [1].

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Expression of histamine degrading enzymes in guinea pig tissues

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Histamine can be inactivated either by histamine N-methyltransferase (HNMT) catalyzed ring methylation or by diamine oxidase (DAO) catalyzed oxidation of the primary amino group. Although the guinea pig has historically been one of the most important model systems for studying histamine function relatively little information was available regarding the expression and localisation of the histamine degrading enzymes in this species. Therefore, we used enzymatic activity measurements and reverse transcription-PCR to study the distribution of DAO and HNMT in the guinea pig. Tissue samples were recovered from two male and two female animals and immediately frozen. Specific enzymatic activities of DAO and HNMT were determined in cleared tissue homogenates using radiometric assay procedures. DAO and HNMT mRNAs were detected by PCR with gene specific primers after synthesizing cDNA from total RNA prepared from the tissue samples. DAO was found to be expressed in the intestine, the liver, and the spleen whereas the enzyme was present in only insignificant amounts in the kidney and in other tissues. HNMT expression was detected in all tissues analyzed with highest expression levels found in spleen, kidney, and small intestine. For both enzymes we found significant individual variation in specific tissue activities. Compared to other species, DAO and HNMT are present in considerably lower amounts indicating a lower requirement for histamine inactivation capacity in the guinea pig.

Regulation of diamine oxidase expression in mammalian cells

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Diamine oxidase (DAO) catalyzes the oxidative deamination of histamine and other diamines. The enzyme is a member of the class of copper containing amine oxidases that possess the active-site cofactor 2,4,5-trihydroxyphenylalanine quinone. In mammals, the major sites of DAO expression are intestine, kidney, and placenta. To learn more about the regulation of tissue-specific expression of DAO we studied the promoter of the porcine DAO gene. Reporter gene assays with fusions of DAO promoter fragments with firefly luciferase cDNA showed that a stretch of ca. 150 bp upstream of the transcription start site is sufficient for high-level expression in porcine kidney epithelial and human colon carcinoma cells. Deletion and mutation constructs of this DAO core promoter region identified several transcription factor binding sites that are also present in the human DAO gene promoter. Positive regulation is mediated by three tandem Ap2 repeats, a Pu-1 site and a Sp1 site, whereas a c-Jun site and an E-pal motif confer negative regulation. Specific binding of regulatory proteins at these sites was confirmed by electrophoretic mobility shift assays. These revealed a similar protein binding pattern in porcine and human cells. Subtle differences in the binding of individual factors and an additional interaction of a protein with the proximal promoter region seen only in human cell extracts might be responsible for the differences in expression levels observed in pig and man.

My Journey with Histamine from the Cardiovascular System to the Brain

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The progress in the therapeutic modulation of various histamine actions were made possible through the discovery and subsequently better understanding of the histamine receptor subtypes (H_1-, H_2-, H_3-, H_4-). In the vascular smooth muscle and endothelium H_1-, H_2- and H_3-receptor subtypes were identified on the molecular level using radioligand binding. The characteristics of H_1-, H_2- and H_3-receptor in the above tissues differ in the quantitative (number of binding sites) and qualitative parameters (affinities of various ligands, regulation by guanil nucleotides, mono and divalent cations, group selective reagents, etc.). Species differences were also observed using the model system of bovine and guinea pig aorta. Histamine H_1- and H_2-receptors also coexist in the myocardial membranes of guinea pig with the significant prevalence of H_1-receptors. These results indicate that the heterogeneity of responses to histamine observed among different species and different type of vascular beds are at least partly due to the differences in the exact localization, functional and molecular properties of all three cardiovascular histamine receptor subtypes. Several groups of new highly selective histamine H_1-receptor ligands from the class of 2-phenylhistamines, histaprodifen derivatives (methyl-, dimethyl- and imidazolylethyl-histaprodifen and pyridylbutylhistaprodifens) are potent but heterogeneous H_1-receptor ligands with diverse (antagonistic and/or agonistic) effects on the molecular level in our model system of aortic H_1-receptor. In the CNS, histamine receptors (H_1-, H_2-, H_3-) were identified also on the rat neonatal astrocytes in primary cultures where the activation of H_1-receptors showed remarkable effect on the neurotrophic activity of astrocytes causing the stimulation of NGF release.
The results of this complex study contribute to the better understanding of the peripheral and central role of the histamine receptors.

Effect of Genistein on Agonist-Induced Airway Smooth Muscle Contraction

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Genistein, a tyrosine kinase inhibitor, blocks antigen-response in a guinea-pig asthma model [1], inhibition possibly involving mast cells, other inflammatory cells, or airway smooth muscle contraction, which we investigated.
In isolated guinea-pig tracheal rings (GPTR) [2], genistein (10-40 μM) had a small relaxant effect and a more marked time- and dose-dependent inhibition of contraction which was highest against histamine. At 40 μM (30 min pre-incubation), inhibition was 91.8±2.9% *v* 5-10 μM histamine (acting *via* H_1-receptor), 45.6±12.6% *v* 0.5-1.0 μM carbachol (*via* m3 muscarinic receptor) and 45.9±14.6% *v* 0.5-3.0 mM NaF(G-protein activator). Inhibition washed off slowly. Compared to genistein, benzalkonium chloride (G-protein G_i-type inhibitor)[3] 1 μM had a similar action, but inhibition was highest against NaF (87.0±4.1%; 51.1±9.2% *v* histamine, 69.2±8.6% *v* carbachol).
Genistein's action was uninfluenced by indomethacin (50 μM), suggesting that prostaglandins are not involved. Methylene blue, a guanylyl cyclase and NO synthase inhibitor, at 20 μM produced a small but significant reduction in genistein's inhibition of the carbachol response, suggesting that NO may be involved in that inhibition. The α-chymotrypsin antagonist, TPCK (500 μM) blocked genistein's action *v* NaF (inhibition down to 13.2±8.4%) and less markedly *v* carbachol, but not histamine. Thus, a trypsin-like enzyme may be implicated in mediating the inhibition by genistein. We have previously shown that trypsin itself produces some contraction at 1-10 μM, while causing relaxation at higher concentrations [2]. Pre-treatment with >10 μM trypsin also inhibits the response of GPTR to the spasmogens used in this study.

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New Ligands For The Histamine H_4 Receptor

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The histamine H_4 receptor (H_4R) was recently cloned and is involved in the chemotaxis of leukocytes and mast cells to sites of inflammation. The selective H_4R antagonist JNJ 7777120 possesses anti-inflammatory properties, suggesting the H_4R is a potential drug target. So far, potent selective H_4R agonists have not been identified. In the present study we therefore evaluated the human H_4R (hH_4R) for its interaction with various known histaminergic ligands. Almost all tested H_1R and H_2R antagonists, including several important therapeutics, displaced less than 30% of specific [^{3}H]histamine binding at concentrations up to 10μM suggesting that these antagonists do not interact with the hH_4R. Most tested H_2R agonists and imidazole-based H_3R ligands exhibit H_4R affinities in the micromolar- to nanomolar-range and exert different intrinsic hH_4R activities, ranging from full agonists to inverse agonists. Interestingly, we identified a high affinity H_4R ligand (K_i = 50 nM) that has a >100-fold selectivity for the hH_4R over the other histamine receptor subtypes. Moreover, this compound potently activates the hH_4R (pEC_{50} = 7.4 ± 0.1, α=1) and this response was competitively antagonized by the selective H_4R antagonist JNJ 7777120 (pA_2 = 7.8). The identification of a potent H_4R agonist is of major importance for future studies to unravel the physiological roles of the H_4R.

Differences in the Binding Characteristics and Intracellular Effects of Specific Histamine H_1- Agonists - Pyridylbutylhistaprodifens in the Cardiovascular Tissue

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We determined the molecular properties of the selective and potent H_1-receptor agonist - derivatives of histaprodifen: o´-pyridylbutylhistaprodifen, m´-pyridylbutylhistaprodifen and p´-pyridylbutylhistaprodifen. All derivatives showed high affinity for the ^{3}H-mepyramine labelled bovine aortic H_1-receptor binding sites with the following order of potency: p´-pyridylbutylhistaprodifen > m´-pyridylbutylhistaprodifen > o´-pyridylbutylhistaprodifen and K_i = 24.2 nM, 37.2 nM and 81.9 nM, respectively. All derivatives were more potent than histamine (K_i = 246 nM) whereas the title compound - histaprodifen has an K_i of 77.6 nM.
Since H_1-receptor agonists are known as activators of G-proteins, the influence of GTP on the binding characteristics was tested. Presence of GTP (100 μM) shifted p´-pyridylbutylhistaprodifen competition binding curve to the left, whereas the competition curve of m´-pyridylbutylhistaprodifen and o´-pyridylbutylhistaprodifen were shifted to the right as expected from the agonists. All derivatives (conc. 10 μM) activate G-proteins as monitored by the rate of GTPγS binding to the bovine aortic membranes. Their rank order of potency: p´-pyridylbutylhistaprodifen > m´-pyridylbutylhistaprodifen > o´-pyridylbutylhistaprodifen correspond well to the rank order of their affinities for the ^{3}H-mepyramine labeled aortic H_1-receptor.
Our data suggest that pyridylbutylhistaprodifens are potent but heterogeneous H_1-receptor agonists as shown also in the functional studies on guinea pig aorta (1). Their diverse effects on the molecular level in our model system correspond with the studies on other histaprodifen derivatives (2).

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Conformational Restriction in the Design of Diamine –Based Human Histamine H_3 Receptor Antagonists.

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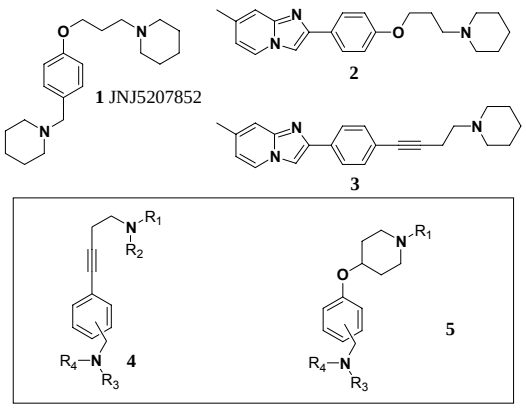
We have previously described several series of diamine-based human histamine H_3 antagonists [1-3] including the simple bis-piperidine JNJ-5207852 (**1**) [4]. Noting the almost identical receptor affinities of the arylether **2** and the arylalkyne **3**, our attention turned to the preparation of conformationally restricted replacements for the ubiquitous piperidinylpropyloxy fragment present in **1** and in many other non-imidazole histamine H_3 antagonists. To this end we examined the effect of replacing the propyloxy linkage with a butynyl linkage (e.g. **4**) and, separately, with a piperidinyloxy linkage (e.g. **5**). In both cases potent and highly selective H_3 receptor antagonists were obtained that exhibit favorable PK/PD and ADME properties together with in vivo efficacy in models of vigilance. The synthesis, SAR and in vivo data for these series will be presented.



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Evidence for Human and Rodent H_3 receptor Dimers: A Cross-linking Study

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The histamine H_3 receptor (H_3R), expressed almost exclusively within the CNS, is a classic GPCR. Alternative splicing of the H_3 receptor gene gives rise to several isoform mRNAs which are differentially distributed within the CNS. There is increasing evidence that many GPCR's function as dimers. We have previously reported immunoblotting evidence in native tissues for the existence of H_3R dimers in the rodent brain (1). Here we provide further new evidence for the existence of both rodent and human H_3R dimers. HEK293 cells were transfected with selected individual FLAGhH_3R or rH_3R isoforms cDNA using the LipofectaminePlus method, harvested 40h post-transfection and homogenised prior to cross-linking using the irreversible cross-linker Bis (sulfosuccinimidyl) suberate (BS_3). Crosslinked proteins were subjected to immunoblotting, using anti-FLAG or our novel specific anti-H_3 receptor antibodies (0.2- 1µg/ml), respectively.

For both the rat and human full length $H_{3\ (A)}$ isoform, increasing concentrations of the cross-linker up to 1mM resulted in increasing amounts of protein species with a size compatible with a dimer (M_r 91,000), with a concomitant decrease in the amount of monomer. Interestingly, under identical conditions, the $rH_{3\ (C)}$ isoform did not appear to crosslink to form a higher molecular weight species. Therefore, we provide further evidence for the existence of rodent and human $H_{3A}R$ dimers, in agreement with our FRET studies on rat recombinant receptors, and immunoblotting data using human brain tissue, respectively. We also report that individual rH_3R isoforms display differential propensity to form dimers, which may indicate that particular shorter isoforms oligomerise as hetero-, rather than homo-oligomers.

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Antiinflammatory, Analgesic and Gastroprotective Effects of the Novel and Selective Histamine H_4-Receptor Antagonist VUF5949

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The histamine H_4 receptor was recently identified in cells of hematopoietic lineage, including eosinophilis, T cells, dendritic cells, basophilis and mast cells, where it can mediate chemotaxis and calcium influx (1). The present study examined the analgesic, antiinflammatory and gastric effects of the novel and selective histamine H_4-receptor antagonist, compound VUF5949 (2), in comparison with indomethacin (IND).
Inflammation was induced in rats by subplantar injection of carrageenan (CARR) into the left hind paw. Paw edema and thermal hyperalgesia were measured immediately before CARR injection and thereafter at 2, 4 and 6 hrs. The development of gastric lesions was assessed 6 hrs after administration of the compounds. “Lesion index” was determined by measuring each haemorrhagic lesion along its greatest length (mm).
VUF5949 (10 mg/kg, subcutaneously,sc) significantly inhibited either the edema (maximal reduction = -79.08 %) or the hyperalgesia (+65.20 % paw withdrawal latency) induced by CARR. The effects of VUF5949 were only evident 2 hrs after CARR injection, while the antiinflammatory and analgesic effects of IND (20 mg/kg, sc) were observed at 2, 4 and 6 hrs. VUF5949 did not induce damage to the stomach at any dose tested (3-30 mg/kg sc), whereas IND provoked gross hemorrhagic lesions in the gastric mucosa (lesion index: 26.06±5.55 mm). VUF5949, given simultaneously with IND, significantly reduced IND-induced gastric lesions (lesion index = 9.40±3.49 mm, maximal inhibition = -63.93%).
In conclusion, our study shows that the histamine H_4-receptor antagonist VUF5949 had antiinflammatory and analgesic activities, combined with marked gastroprotective effects. These data suggest a potential role of histamine H_4 receptors in inflammation and in gastroprotection.

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Cross-Interaction between Par-Activation and Tyrosine Kinase in the Modulation of Rat Ileum Smooth Muscle Contraction (RIMC)

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Serine protease, tyrosine kinase and protein phosphorylation might interact to regulate smooth muscle activity [1-4]. We have studied the effect of TPCK (α-chymotrypsin inhibitor), genistein (tyrosine kinase inhibitor) and cyclosporin-A, CsA (type 2B protein phosphatese inhibitor) on the relaxation of NaF- and carbachol-induced RIMC by NECA (adenosine A_2 agonist), α–chymotrypsin (PAR-2 agonist), genistein and isoprenaline (β_2–agonist, the relaxation of which is antagonised by phosphatase 2A inhibitor).
RIMC was studied as previously described [3]. NaF or carbachol was added to one ileum preparation once before and once after a 15 min incubation with a given concentration of NECA, α–chymotrypsin, genistein or isoprenaline. TPCK or CsA were administered together with NECA, α–chymotrypsin, genistein or isoprenaline for 15 min. The results were expressed as percentage inhibition of the stimulant-induced contraction.
NaF (10^{-2}M) and carbachol (0.5 μM) induced a 50% contraction above basal level. NECA, α–chymotrypsin, genistein and isoprenaline produced dose-related relaxation of stimulated contraction. TPCK (10^{-4}M) markedly and significantly reversed the relaxating effect of α–chymotrypsin (20 μg ml^{-1}).and genistein (1μ M), but not that of NECA (2 μM) or isoprenaline (1 μM). CsA had no noticeable effect. The effect of α–chymotrypsin and genistein was markedly and significantly reversed by aminoguandine (NO synthase inhibitor).
The present results suggest that there is cross-interaction between PAR activation and tyrosine kinase in relation to RIMC, and that nitric oxide might be involved.

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Fluorescent Benzofuran H_3 Antagonists with Sub-Nanomolar Potency

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Fluorescent ligands have the potential to help probe the interaction of histamine H_3 receptors with ligands and other proteins. H_3 receptors are found on pre-synaptic nerve terminals throughout the central and peripheral nervous system, where they modulate the release of different neurotransmitters, with agonists generally reducing, and antagonists generally increasing release. To generate additional tools to help researchers probe this pharmacology, fluorescent compounds with sub-nanomolar potency at human and rat H_3 receptors were designed in a benzofuran series of H_3 antagonists by making the fluorophore an integral element of the structure; it was found that the 4-cyanophenyl moiety of ABT-239 (4-{2-[2-(2(R)-Methyl-pyrrolidin-1-yl)-ethyl]-benzofuran-5-yl}-benzonitrile) could be replaced with a variety of heterocyclic fluorophores. The product molecules are exemplified by 2-[2-(2(R)-methyl-pyrrolidin-1-yl)-ethyl]-benzofuran-5-ylmethyl-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine, which has Ki values of 220 pM and 650 pM at human and rat H_3 receptors, and 5-dimethylamino-naphthalene-1-sulfonic acid 2-[2-(2(R)-methyl-pyrrolidin-1-yl)-ethyl]-benzofuran-5-yl-amide, with Ki values of 5.9 nM and 8.9 nM at human and rat H_3 receptors. It is anticipated that such compounds could find use as research tools to probe the environment of the receptor binding site, or probe protein-protein interactions between H_3 receptors and appropriately labeled effector proteins or co-expressed receptors.

Histamine and its metabolite N-alpha-methyl histamine accelerate ulcer healing in rats: role of H3 receptors, sensory nerves and TGFα

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Background: The selective agonists of H_3-receptors protect the gastric mucosa against acute damage from various noxious agents in rat (Morini G et al. Br J Pharmacol 2002, 137, 237-244). However, the role of histamine and its metabolite, N-alpha-methyl histamine (Nα-MH), produced in *H. pylori*-infected gastric mucosa, in the process of ulcer healing has not been studied. The aim of the present study was; 1) to assess the effect of histamine and N-alpha-methyl histamine (Nα-MH) in the presence of selective H_2 (ranitidine) and H_3 (clobenpropit) antagonists on the ulcer healing in rat; 2) to study the effect of histamine and Nα-MH on the release of gastrin and expression of transforming growth factor alpha (TGFα) and hypoxia-inducible factor-1 (HIF-1), two important protective factors, in rats with intact and deactivated sensory nerves with neurotoxic dose of capsaicin.
Methods: Gastric ulcers were induced in rats by acetic acid method. Rats with ulcers and intact or capsaicin deactivated sensory nerves were divided into following treatment groups: **A**) vehicle (saline); **B**) histamine (2 mg/kg i.p./day); **C**) Nα-MH (2 mg/kg i.g./day); **D**) ranitidine (30 mg/kg s.c./day) + histamine (2 mg/kg i.p./day), **E**) ranitidine (30 mg/kg s.c. /day) + Nα-MH (2 mg/kg i.g./day); **F**) clobenpropit (10 mg/kg s.c./day) + histamine (2 mg/kg i.p./day). At day 9 after ulcer induction, the rats were sacrificed, the area of gastric ulcers was determined by planimetry and gastric mucosal blood flow by H_2 gas clearance method. The gastric mucosal biopsy and blood samples were taken for the determination of mRNA and protein expression of TGFα by RT-PCR and Western blot, respectively, and for RIA of serum gastrin level.
Results: Treatment with histamine and NαMH accelerated ulcer healing and this effect was accompanied by the increase of gastric mucosal blood flow at the ulcer margin and the rise in serum gastrin level. Application of histamine and NαMH increased the mRNA and protein expression of TGFα in the gastric mucosa, but it had no effect on HIF-1alpha mRNA expression which was significantly increased after ulcer induction as compared to the intact gastric mucosa. Treatment with H_3-receptor antagonist (clobenpropit) combined with the application of histamine or NαMH significantly attenuated the increase in gastric mucosal blood flow and serum gastrin leading to delay of ulcer healing. Inactivation of sensory nerves also attenuated significantly the increase in ulcer healing, mucosal blood flow and serum gastrin increase induced by histamine and NαMH. In contrast, treatment with H_2 antagonist did not affect the accelerated ulcer healing induced by histamine and NαMH.
Conclusion: Histamine and NαMH accelerate ulcer healing and this effect is mediated mainly by H_3 but not H_2 receptors. This ulcer healing effects of histamine and NαMH is mediated,at least in part, by increased expression of TGFα and release of gastrin and activation of sensory nerves.

Dualistic H_1-mediated Effect of Histamine on the Isolated Human Intestine

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Histamine (HA) contracts gastrointestinal smooth muscle cells from both animals and humans through the activation of H_1 receptors. The recent discovery of the H_4 receptor subtype, causing a rise in the intracellular Ca^{2+} levels and then representing a potential mechanism of contraction, prompted us to re-investigate the effects of HA on the human intestinal motility. The effects of HA were tested on intestinal strips, taken from human colonic or ileal specimens excised during intestinal surgery. In such preparations, the effects of HA and of HA receptor antagonists were evaluated for their ability to modify the basal tone and spontaneous motility.
HA (10 nM-100 μM) induced a transient relaxation, followed by a tonic contraction of longitudinal muscle preparations from both ileal and colonic strips. The effects of HA were refractory to H_2, H_3 and H_4 receptor blockade by famotidine (1 μM), FUB649 (1 μM) or compound J&J 7777120 (1 μM), respectively, but counteracted by the H_1-receptor antagonists, mepyramine and chlorphenyramine, in the same range of concentration (1 nM-1 μM). The effects of HA could not be reproduced by the specific H_1-receptor agonist, 2-(3-trifluoromethylphenyl)histamine. Tetrodotoxin (1 μM), propranolol (1 μM) and L-NAME (100 μM) did not cause any significant modification of the HA-induced inhibitory response, excluding the involvement of inhibitory adrenergic or NANC neurons or the activation of nitric oxide (NO)-producing cells.
The response of the human intestinal muscle to HA is related to the activation of H_1 receptors, which mediate both excitatory and inhibitory effects in both colon and ileum. No evidence of functional histamine H_4 receptors in the human intestine could be provided. The so far unexplored H_1-mediated inhibition is independent of substances released from the intramural nerves and of NO spillover from cells resident into the intestinal wall. Other autacoids (inhibitory prostaglandins and/or purinergic transmitters), or the activation of K^+ fluxes in muscle cells could be involved.

Distinctions and Contradistinctions between Antiobesity Histamine H_3 Receptor (H_3R) Antagonists Compared to Cognition-Enhancing H_3 Receptor Antagonists

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We identified some H_3R antagonists with antiobesity properties [1,2] while others enhance cognition [3,4] although no mechanism reliably differentiates these activities. One proposal states that inverse agonist activity is required for antiobesity effects [5]. In our experiments, inverse agonist activity was not sufficient for weight loss; A-631972, a potent, selective and lipophilic H_3R inverse agonist was inactive in mouse diet-induced obesity. Likewise, we found no major differences between potencies or efficacies of key compounds at human H_3R isoforms. A third hypothesis was based upon variable activation of c-Fos in hypothalamic nuclei [6]. Preliminary studies comparing A-331440, A-423579, ciproxifan and ABT-239 reveal qualitative differences in c-Fos activation in various brain regions. Specifically, doses of H_3R antagonists that increased c-Fos expression in paraventricular nucleus, a hypothalamic brain region key to feeding, were efficacious in antiobesity feeding models. Conversely, activation of c-Fos in hippocampus or prefrontal cortex may be important for pro-cognitive behavior, as increased c-Fos expression in these regions was observed with precognitive H_3R antagonists. Therefore, differential expression of c-Fos by diverse H_3R antagonists may provide a mechanistic biomarker of distinct pharmacological activities.

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Interactions between the histaminergic and angiotensinergic systems in the central cardiovascular regulation in rats

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Histamine, acting centrally as a neurotransmitter, influences the central cardiovascular regulation. Both a central injection of exogenous histamine and an increase in endogenous histamine concentrations, after inhibition of histamine N-methyltransferase activity, lead to the pressor effect in normotensive rats. The action is due to an activation of the sympathetic system and secretion of arginine vasopressin. Previous papers clearly demonstrate interactions between the histaminergic and opioidergic, muscarinic cholinergic and noradrenergic systems in the central cardiovascular regulation. Since angiotensinergic neurones are able to influence cardiovascular centre function, present studies were undertaken to determine possible interactions between the histaminergic and angiotensinergic systems in the regulation of the circulation. Experiments were performed on male Wistar-Kyoto rats under ethylurethane anaesthesia (1.25 g/kg; intraperitoneally). Histamine (20 µg/5 µl; intracerebroventricularly [icv]) caused rapid, lasting 20-25 min, increase in mean arterial pressure (MAP) and heart rate (HR), maximally up to 22% and 30% of the initial values, respectively. Captopril (0.5 µg/5 µl; icv) – angiotensin convering enzyme inhibitor, and both angiotensin type 1 (AT1) and type 2 (AT2) receptor antagonists ZD 7155 (3.0 µg/5 µl; icv) and PD 123319 (2.0 µg/5 µl; icv), respectively, inhibited histamine-induced changes in MAP, whereas there were no significant differences in HR. On the other hand, neither captopril (0.1, 0.5 µg/5 µl; icv) nor angiotensin receptor antagonists – ZD 7155 (0.5, 1.0, 3.0 µg/5 µl; icv) and PD 123319 (0.5, 1.0, 2.0 µg/5 µl; icv), influence cardiovascular system function in control groups. In conclusion, we demonstrate for the first time functional interactions between the histaminergic and angiotensinergic systems in the central cardiovascular regulation in normotensive rats. Our studies show an involvement of the central AT1 and AT2 receptors in the effect.

Influence of Centrally acting Amitriptyline and Citalopram on Histamine-induced Cardiovascular Effects in Rats

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Histamine, acting peripherally via H1 and H2 receptors, induces a direct vasodilatatory effect leading to hypotension. Our previous studies demonstrate that tricyclic antidepressants inhibit compound 48/80-induced secretion of histamine. Moreover, amitriptyine (AMI) and citalopram (CIT) reduce an increase of plasma histamine levels after its intravenous injection. The action is accompanied by inhibition of histamine-induced decreases in mean arterial pressure (MAP) and pulse pressure (PP), without influence on heart rate (HR). The effect can be mediated both peripherally and centrally. The aim of the study was to examine an influence of centrally acting AMI and CIT on cardiovascular effects elicited by intravenous bolus injection of histamine. Studies were carried out in anaesthetised male Wistar rats. In the saline pre-treated group, bolus injection of histamine (10 μg/kg; iv) transiently decreased MAP and PP by 44.5% and 55.3%, respectively, with no influence on HR. Pre-treatment with AMI (2, 5 μg/5 μl; icv), but not with CIT (1, 3 μg/5 μl; icv), given 20 min or 1 h earlier, inhibited histamine-induced changes in MAP and PP. Interestingly, in the groups pre-treated with AMI (5 μg/5 μl; icv) and CIT (1, 3 μg/5 μl; icv), the depressor effect elicited by histamine was accompanied by an increase in HR. In the control iv saline-treated groups, AMI and CIT had no effect on MAP, PP and HR. Results demonstrate that AMI, but not CIT, acting centrally, influences vascular resistance regulatory mechanisms in rats. Moreover, both agents are able to affect the reactivity of the cardiovascular centre to hypotensive stimuli, since the depressor effect was accompanied by, probably reflex-mediated, tachycardia. Our results show that tricyclic antidepressants can influence histamine-induced cardiovascular effects not only peripherally, but also centrally.

First evidence of histamine (H) release involved in human reflux disease

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The incidence of gastroesophageal reflux disease and its complications are increasing in the Western world. This study investigated ex vivo histamine (H) secretion from normal human esophageal and normal cardiac mucosa with respect to the acidifying effect of hydrochloric acid, imitating gastric reflux.
42 vital biopsies from 6 patients with normal esophagus and normal cardia were taken for ex vivo H release (HR) during 4 hours of mucosa oxygenation. Spontaneous HR was recorded at different pH levels (pH 7, 5 and 4) of the culture medium, adjusted by addition of HCl. HR [median±SD, effective HR: ng/mg ww] as well as total tissue H content [ng/mg ww] were determined by established methods. Tissue H content of squamous mucosa from esophagus (14.7±7.8) and gastric mucosa from cardia (15.3 ± 12.0) were not statistically different.
Interestingly, gastric mucosa showed a clearly higher spontaneous HR than esophageal mucosa, 6.9 ± 2.2. *vs* 1.0 ± 0.5 at pH 7. Acidification of the culture medium induced significantly increased rates of spontaneous HR from both gastric and squamous mucosa (pH 5: 31.6 ± 2.0 *vs* 22.5 ± 1.5, $p < 0.01$ and pH 4: 35.7±0.9 *vs* 31.1±1.9, $p < 0.01$).
However, stimulation of biopsies with anti–IgE (1/80 dilution, Sigma) at pH 7 did not provoke a significant HR neither from gastric nor from squamous mucosa (2.4±0.5 vs 2.2±0.4).
Normal gastric and esophageal mucosal tissue was found to have similar amounts of at pH 7. However, cardiac mucosa shows higher rates of HR than esophageal mucosa. Interestingly, a pH dependent increase of spontaneous HR was found in both tissue types. Thus, acidic gastric reflux is concluded to provoke a non-immunologically induced type of HR, which may be involved in symptoms and patho-histological sequelae of reflux disease, since H exerts proinflammatory, immunosuppressive or mitogenic effects.

Gut mucosal histamine release (HR) in response to polyamines is different in patients with colorectal adenoma and controls: Implications for colorectal adenoma growth?

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One possible connection between polyamines and colorectal carcinogenesis may be the enhancement of gut epithelial cell proliferation. Since histamine may stimulate gut epithelial proliferation, this study investigated the influence of putrescine, spermidine and spermine on the rate of HR from patients with colorectal adenoma and normal colorectal mucosa.
152 vital biopsies from the lower gastrointestinal tract of 18 persons with colorectal adenoma and from 18 controls were incubated in an oxygenated culture medium for 240 minutes (transportable biopsy mucosa oxygenator; INTESTINO-DIAGNOSTICS, Erlangen). The biopsies were incubated with putrescine, spermidine and spermine (10^{-3} mol/l), while anti-IgE (1/80 dilution) served as positive control. HR was measured by ELISA and its net release is given as ng/mg ww $\pm$ SD.
In adenoma patients all three polyamines induced a significantly increased rate of HR from the biopsies (putrescine 7.3$\pm$9, $p<0.005$; spermidine 5.6$\pm$6, $p<0.05$; spermine 4.4$\pm$6, $p<0.005$) compared with the spontaneous HR (1.5$\pm$1). When comparing adenoma patients with controls, a striking difference in the rates of HR can be obtained between both groups. While putrescine induced also a significantly increased HR in controls (6.8$\pm$7, $p<0.005$), spermidine (2.1$\pm$2) and spermine (2.3$\pm$2) failed to evoke a significantly different HR compared to the spontaneous one (0.9$\pm$0.8). Anti-IgE also induced a significant HR in controls (3.7$\pm$1, $p=0.009$), being lower than the HR induced by putrescine.
The observation that all three polyamines induced a significant rate of HR in adenoma patients was a surprising finding, not yet reported before. While putrescine-induced HR may also be caused by a competitive inhibition of diamine oxidase, spermidine- and spermine-induced HR may indicate an non-immunologic type of HR, connecting polyamines via HR with colonic epithelial cell proliferation.

Non-immunogically induced histamine release (HR) of vital biopsies of the human gut by stimulation with polyamines

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In the human gut histamine (H) mediates symptoms of food allergy. Additionally, H has a mitogenic effect, being important for colorectal carcinogenesis. The aim of this study is to investigate if putrescine, spermidine and spermine induce HR in vital intestinal biopsies.
For this study 284 biopsies from the lower gastrointestinal tract of 76 patients of a control group (CG), a group of patients with gastrointestinally-mediated allergy (GMA) and a patient group with colorectal adenoma (AG) were examined. The biopsies were kept vital by incubation in a physiological medium at 37°C and pO_2=85-95 mmHg and were stimulated with putrescine, spermidine and spermine (1mM). The spontaneous HR (SHR) served as negative control and anti-IgE (1/80 dilution) as positive control. H was measured by ELISA and kinetics as well as the effective HR given as ng/mg wet weight ± 95% confidence interval.
The polyamine putrescine had in all of the three patient groups the most powerful effect on the HR. The effective HR was in AG (7,36±4,91) significantly raised (p=0,003) compared to SHR (1,52 ± 0,7). In GMA the effective HR (10,42±10,70) was highly significantly (p<0,0001) enhanced compared to SHR (1,03± 2,81). Putrescine induced HR reached in the CG (6,89±4,17) statistical significance (p=0,006) compared to SHR (0,99±0,42). The kinetics confirm this observation.
The observation that putrescine induced a significant rate of HR in all three groups was a surprising finding, not yet reported before. For patients with unspecific food allergy symptoms these results suppose that a putrescine-rich alimentation could cause their symptoms, especially in patients with H intolerance. High consumption of meat is a known risk factor for colorectal carcinogenesis, which may be due to the high content of polyamines inducing non-immunologically mediated HR and therefore enhancing epithelial cell proliferation.

Histamine in Lager Beer

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Last year we reported histamine (His) in wine. The aim of the present study was to assess whether His and other biogenic amines can be measured in commercially available German lager beers and whether they influence their taste.
8 bottled test samples were purchased from supermarkets and 4 samples of beer on draught were drawn in typical German bars. Type of beer, alcohol, and ingredients like total protein and sugar were documented. Immediately after opening the bottles, freshly drawn beer was tested using standardized sensitivity assessment methods by 10 educated test persons. The reference test samples were diluted in normal tap water (0-10 mg/l His-HCl). His and other biogenic amines like tyramine, cadaverine, phenylethylamine, putrescine, spermidine and agmatine were measured by fluorimetric detection after HPLC and two-dimensional thin-layer-chromatography.
The mean His concentrations were 0.5 mg/l (range 0.05–1.3) in the bottled and 0.8 mg/l (range 0.6–2.5) in the open lager beers, respectively. The other biogenic amines could also be detected depending on both the method of brewing and brewery location. Putrescine, cadaverine, tyramine and agmatine could be identified, and some of them showed higher concentrations than His. The mean threshold for reference His in normal tap water was 2 mg/l ±0.9 (SDM, n=10). Sensitivity criteria such as pharyngeal irritation, tingling tongue, swelling of mucosal tissues in the mouth were defined to be characteristic for the “specific” taste of the biogenic amines. No positive correlation was found between His/biogenic amine concentration in beer and the sensoric sensation (R=0.6; ranking of intensity of biogenic amines in 12 lager beer samples).
As other ingredients, such as alcohol, protein and sugar, reveal interfering influences it can be concluded that His and other biogenic amines cannot be tasted specifically in German lager beer.

The Role of Mast Cells in Allergic Disease and Innate Immunity

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Mast cells have been studied largely because of their role in IgE-dependent allergic diseases. Mast cells are unique in that they alone express abundant numbers of high affinity receptors for IgE (FcϵR1) on their cell surface. Multivalent binding of allergen to FcϵRI-bound IgE initiates multiple signalling cascades that lead ultimately to release an array of potent inflammatory mediators through degranulation with release of preformed mediators such as histamine, the generation of inflammatory lipids from arachidonic acid, and the production of numerous Th2-type cytokines and chemokines. Nonetheless, the benefit of the ensuing inflammatory reaction to the host remained an enigma. However, recent studies now indicate that mast cells mount an effective protective inflammatory response against bacterial and parasitic infections most likely via the pathogen-recognizing Toll-like receptors (TLRs). Mast cells express TLRs and the specific signaling components that are known to mediate responses to TLR ligands such as lipopolysaccharides and peptidoglycans. In addition, the abundance of mast cells in skin, airways, gut, and body cavities means that these cells are strategically located as a first line defense mechanism against acute infections. Interestingly, the TLR ligands do not readily induce degranulation and production of inflammatory lipids but they do induce synthesis of inflammatory cytokines and markedly potentiate production of these cytokines by antigen. We will discuss recent developments in the field, the signalling mechanisms that account for the synergistic responses to FcϵRI and TLR ligation, and the clinical implications for allergic disease.

Comparison of Nociceptin- and Compound 48/80-Induced Histamine Release After a Single, Intracerebroventricular Administration of the Compounds.

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Nociceptin (NC), the 17-amino acid neuropeptide was recently identified as the endogeneous ligand for the opioid-like (ORL1) receptor. At the cellular level, NC has much in common with the classical opioids; however functional studies are revealing distinct action of this peptide.

In our previous work, NC, administered in a single intracerebroventricular (i.c.v.) injection produced a surplus of histamine (HA) in the cerebrospinal fluid (CSF), suggesting a release of HA from brain cells [1]. The aim of present study was to determine the cellular origin of it.

We measured the HA and 5-HT (serotonin) contents in the CSF and the plasma of rats after a single i.c.v. injection of NC and 48/80, following our previous protocol and using the isotopic micro assay for HA and the liquid chromatographic method for 5-HT [1,2].

48/80 revealed remarkable enhancements of plasma and CSF HA and 5-HT contents. NC did not produce enhancements in 5-HT contents but increased the CSF level of HA to a marked extent.

48/80 degranulates perivascular mast cells (MCs) secreting HA and 5-HT and increases the permeability of the blood-brain barrier (BB) [3]. The increased HA and 5-HT contents of plasma, paralleled to that of the CSF by a single i.c.v. administration of 48/80; appear to confirm the above statement. The NC-induced HA surplus in the CSF, without a 5-HT excess, shows a neuronal release but does not exclude an MC secretion, differed from 48/80-induced degranulation.

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Mast Cell Density in Secretory Meningiomas

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The secretory meningioma is relatively rare subtype of meningioma, only 1.1% in our institution with predominance of female patients 6:1. The unique features, both concerning clinical and pathological aspects of secretory meningioma have not yet been completely understood.
This study analysed the effects of histological characteristics on radiological evidence of surrounding tumour oedema which occur more frequently in this subtype of meningioma.
14 cases (12 female) of secretory meningiomas were identified out of >1300 meningiomas from our institution over the last 25 years. Paraffin embedded tumors were retrospectively analysed by histological and immunohistochemical staining for their clinical and pathological features. Mast cells were visualized and counted with the CD-117 immunoreaction. Immunostaining was performed for vascular endothelial growth factor (VEGF), histamine, serotonine, substance P, different cytokeratines and SM-actin.
The main morphological finding was the occurrence of a significantly higher proportion of mast cells in secretory meningiomas compared to non-secretory meningiomas: mean 2.43 (range 0.020-5.60) vs. 0.27 (range 0.0–2.0) cells per 0.0575 mm^2 ($p=0.0001$, Mann-Whitney-U-test). Mast cells were identified in close relation to vessel wall proliferation. Electron microscopy of such proliferated vessels revealed increasing vacuolisation in the peripheral layers, constantly adjacent to small oedematous areas, in which the mast cells were often freely floating. A significantly positive coarse-grained intracellular VEGF-staining was found in mast cells of secretory meningiomas. Furthermore, the pseudopsammoma bodies characterizing those tumours are surrounded by cells that react vividly with cytokeratins (CK). Only CK 20 was regularly absent. Only serotonine staining was positive at the attempts to characterize mast cells and secretory products by a panel of tissue hormones (serotonine, histamine and substance P).
We present a hitherto not noticed pathological finding that could shed new light upon this rare entity. VEGF and serotonine expression, pericytic vessel proliferation, oedema formation of secretory meningiomas and the newly described occurrence of mast cells may well be correlated. Further analysis of these features is difficult because these tumours are rare.

Systemic and local effects of cannabinoids on histamine release in the rat brain

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The histaminergic system provides a major excitatory source to the entire brain and affects learning and memory. Activation of cannabinoid receptors in the CNS induces several behavioural effects including alteration of cognition, locomotion and pain perception. As drug addiction may be considered a form of learning, we are interested in understanding how histamine (HA) is involved in addiction mechanisms. Using the dual probe microdialysis technique in freely moving rats we previously showed that infusion of selective CB1 receptor agonists in the tuberomammillary nucleus (TMN) significantly increased HA release from the TMN and the ipsilateral nucleus basalis magnocellularis (NBM). This effect was abolished by co-infusion of AM251 a selective CB1 antagonist together with the agonists (Cenni et al., 2005 Abstr. Neurosci Meeting). Here we report that:
1) Administration of the CB1 agonist methanandamide (mAEA; 1 μM) for 60 min in the TMN increases HA release in another brain region, the striatum, by about 120% (spont. release=0.054±0.01 pmol/15min) and approx. 50% in the TMN (spont. release=0.09±0.03 pmol/15min; n=5).
2) Systemic (3mg/kg i.p) administration of the selective CB1 agonist ACEA, that more closely mimics the use of Cannabis in humans, increased HA release in the TMN by approx. 80% (spont. release= 0.18±0.06 pmol/15min; n=9). In the striatum HA levels increased by about 120%, (spont. release= 0.138±0.085 pmol/15min; n=5), whereas in the NBM the increase did not reach statistical significance (spont. release=0.148±0.03 pmol/15min; n=4). I.p. injections of saline did not modify HA spontaneous release in the TMN, striatum and NBM (n=4). At present we do not known if the effect of cannabinoids on histaminergic neurons is indirect and decreases an inhibitory tone, and whether other cells are implicated in the circuit. This is one of the next steps in our research.

Blockage of Histamine H1 Receptor Attenuates Social Isolation-Induced Disruption of Pre-Pulse Inhibition: A Study in Histamine H1 Receptor Gene Knockout Mice

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Histaminergic neurotransmission has been implicated in the pathophysiology of stress-related psychiatric diseases. Although several atypical antipsychotics are potent H1 anagonists, the clinical significance of interaction between atypical antipsychotics and H1 receptors is still unknown. In this study, we investigated the effects of H1 receptors blockage on social isolation-induced behavioral changes in H1 receptor gene knockout (H1KO) mice and their wild-type (WT) mice. Both H1KO and their WT mice were subjected to four-week social isolation rearing after weaning (21 postnatal days). After the 4-week isolation period, mice behavioral changes were evaluated using behavioral tests. Locomotor activity in home cages was significantly lower in isolation-reared WT mice than in socially-reared WT mice. However, no change in locomotor activity was observed between isolation- and socially-reared H1KO mice. Social isolation significantly impaired prepulse inhibition of startle response (PPI) in WT mice, but not in H1KO mice. In addition, social isolation significantly impaired spatial learning and memory in WT mice, but not in H1KO mice. Furthermore, H1KO mice treated methamphetamine (METH) showed no enhancement in isolation-induced disruption of PPI. A neurochemical study revealed that isolation-reared WT mice had significantly lower dopamine (DA) levels and slightly increased DA turnover in the cortex than socially-reared WT mice. Conversely, isolation-reared H1KO mice showed significantly higher DA contents as compared to socially-reared H1KO mice. The results of our study indicate that blockage of H1 receptor-mediated neurotransmission attenuates social isolation-induced behavioral changes and that the therapeutic effects of atypical antipsychotics are mediated, at least in part, by interaction with H1 receptors in the brain.

Histamine H_3 and GABAa receptors modulate the activity of histamine neurons.

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Histamine (HA) neurons located in the tuberomammillary nucleus (TMN) project to the whole brain and control arousal, wakefulness and cognition. Their activity is regulated according to the behavioural state by GABAergic inhibition. Furthermore, H_3-receptors are potential targets for arousal control and treatment of sleep-wake disorders (Passani et al., 2004). A dual probe microdilaysis technique was used in freely moving Sprague-Dawley rats to infuse compounds the TMN and determine HA contents both in the TMN and nucleus basalis magnocellularis (NBM). In the NBM activation of H_1 receptors modulate ACh spontaneous release in the frontal cortex [1] and ameliorates memory in the object recognition test [2]. Administration of bicuculline (10 μM) in the TMN for 75 min increased by approximately 100% HA release in the TMN (spont. release = 0.18 ± 0.04 pmol/15 min; flow rate 2μL/min; n=2), whereas no significant increase was observed in the NBM (spont. release=0.24 ± 0.045 pmol/15 min, n=2). Administration of 300 nM thioperamide in the TMN increased histamine release by about 200% from the TMN (spont. release = 0.087±0.01 pmol/15min; n=3) and by approximately 125% in the NBM (spont. release=0.09 ± 0.04 pmol/15 min; n=4). H_3 receptor antagonists modulate HA release in the NBM also when applied locally: 300 nM thioperamide in the NBM increased HA release by approx. 110% (spont. release = 0.2 ± 0.013 pmol/15 min; n=7; flow rate 2μL/min).
These results may have implication for novel therapeutic approaches of cognitive disorders.

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Effects of betahistine on ACh and histamine release from the rat brain

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Nucleus basalis magnocellularis (NBM) neurons provide cholinergic innervation to the cortex, and play a pivotal role in cognition. Intra-NBM administration of H_3 antagonists increased cortical ACh release; this effect was blocked by H_1 antagonists [1]. Presumably, H_3 autoreceptor blockade releases endogenous histamine that impacts on postsynaptic H_1 receptors. This study investigates betahistine effects on ACh and histamine release from the rat brain. Betahistine is both a partial H_1 agonist, and an H_3 antagonist. Male Wistar rats (225-275 g) were anesthetized and implanted with two microdialysis probes, in the NBM to measure histamine output, and in the ipsilateral cortex to measure ACh release. Microdialysis was performed 24 hours after surgery. Perfusion rate was 2 µl/min and 30 µl fractions were collected. ACh was determined by HPLC-electrochemical detection, and histamine by HPLC-fluorimetric detection. Perfusion with Ringer solution containing 100 mM KCl for 15 min stimulated cortical ACh release by about 100 % (basal release: 1.2 ± 0.1 pmol/10 min, n=3), thus cortical ACh release depends on NBM impulse flow activity. Introduction of 100 µM betahistine into the NBM-perfusing medium for 30 min increased significantly cortical ACh release by about 200 % (basal release: 0.9 ± 0.2 pmol/15 min, n=3). Introduction of 100 µM betahistine into the NBM-perfusing medium for 30 min failed to increase NBM histamine release (basal release: 0.17 pmol/15 min, n=7). Since NBM administration of betahistine released cortical ACh, but not NBM histamine, its effect may be attributed to H_1- stimulation, and not H_3-receptor blockade. Nevertheless, cholinergic modulation by betahistine may have functional relevance, as H_1 receptor agonists improved rat performances in the object recognition test [2].

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Differential CNS Expression and Functional Activity of Multiple Human H_3 Receptor Isoforms.

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The existence of multiple human H_3 receptor splice isoforms raises the possibility that isoform specific properties including ligand affinity, signal transduction coupling, and brain localization may differentially contribute to observed *in vitro* and *in vivo* effects of H_3 receptor antagonists. We have confirmed by RT-PCR the expression of full-length H_3(445) mRNA as well as H_3(365) splice isoform mRNA in multiple human brain regions including the amygdala, caudate nucleus, substantia nigra, thalamus, and cerebellum with the H_3(445) isoform more abundant in all regions than the H_3(365) isoform. The H_3(413) was much less abundant and detected only in the caudate nucleus and amygdala whereas the H_3(329) isoform was only detected in the amygdala. When expressed in C6 cells, H_3(445), H_3(415), H_3(365), and H_3(329) exhibit high affinity binding to the agonist ligand [^{3}H]-N-α-methylhistamine with respective K_d values of 0.45, 0.93, 0.32, and 0.94 nM, respectively. In order to determine if these isoforms functionally couple to signaling events, the receptors were co-expressed with the chimeric Gαqi5-protein and tested for their ability to couple to calcium mobilization as determined by Fluorometric Imaging Plate Reader (FLIPR). The H_3R agonist R-α-methylhistamine activated calcium mobilization in cells expressing the H_3(445), H_3(415), and H_3(365) with respective EC_{50} values of 1.3, 4.2, and 0.8 nM, respectively. However, the response to R-α-methylhistamine in cells expressing H_3(365) was less than that for H_3(445) and H_3(415) and no response was elicited in cells expressing the H_3(329) isoform. These studies indicate that multiple functional H_3R isoforms are expressed in human brain that appear to possess differential signal transduction coupling that may influence the therapeutic outcome of H_3 receptor antagonists. *Funded by Abbott Laboratories.*

Differences In Pharmacological Properties of Histamine H_3 Receptor Agonists and Antagonists Revealed at Two Human H_3 Receptor Isoforms.

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Multiple splice isoforms of the histamine H_3 receptor exist, suggesting the possibility of differential H_3 receptor ligand activity at these receptors. We compared the binding and functional (agonist, antagonist, and inverse agonist) potencies of selected H_3 receptor ligands at the human H_3(445) and H_3(365) receptors. The agonist ligand [^{3}H]-N-α-methylhistamine demonstrates high affinity for both receptors with respective K_d values of 0.45 and 0.32 nM. The binding affinities of agonists and antagonists for the H_3 receptor isoforms showed good correlation (r^2 = 0.81). However, agonist binding tended to correlate better (r^2 = 0.89) than antagonist binding (r^2 = 0.73), albeit with overall higher agonist potencies (from 2-10-fold) for H_3(365) and slightly higher antagonist potencies (approximately 2-fold) for H_3(445). Agonist potencies for activation of GTPγS binding by H_3(445) and H_3(365) correlated well (r^2 = 0.94), again with higher potencies noted at H_3(365), which exhibited lower levels of activated GTPγS binding than H_3(445). Antagonist potencies for inhibition of agonist-activated GTPγS binding showed good correlation (r^2 = 0.89). H_3(365) was more constitutively active than H_3(445), where full inverse agonists reduced basal activity by approximately 35% compared to 20% at H_3(445). Inverse agonist potencies correlated well (r^2 = 0.89) with the potencies tending to be higher (from 3-8-fold) at H_3(445). These studies indicate that binding and functional potencies of H_3 receptor agonists and antagonists correlate well for H_3(365) and H_3(445). However, the greater constitutive activity of H_3(365), higher agonist binding and functional potencies at the H_3(365), and greater efficacy, but lower potency, of inverse agonists at the H_3(365) may all impact the pharmacological activity *in vitro* and *in vivo* of H_3 receptor antagonists. *Funded by Abbott Laboratories.*

In Vivo Effect of Sodium Cromoglycate on the Reduction of Hypothalamic Histamine Levels in Hyperthyroid Rats

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Brain histamine is localized in mast cells (MCs) and neurons. Reduction in hypothalamic histamine (HHI) levels has been observed upon systemic administration of L-thyroxine (T_4) or C48/80. This study aimed at investigating the association between hypothalamic MCs and reduced HHI levels in hyperthyroid rats.

Male Wistar rats of 300-350g were divided into 7 groups of 3-14 animals each. Group 1 received no treatment. Groups 2-3 were injected with saline for 2h or qd x5d, respectively. Groups 4-7 were subdivided into A and B. Groups 4A-6A were injected s.c with 0.25mg/Kg T_4 x7d, 10d or 14d respectively, while groups 4B-6B received saline accordingly. Groups 7A-B were treated s.c. qd x10d with 30mg/Kg sodium cromoglycate (SCG) 45min prior to T_4 or saline, respectively. All animals were sacrificed 2h after the last dose. The hypothalami were dissected out, the HHI was quantified fluorometrically and expressed as the mean$\pm$SEM of the % of their respective controls. Statistical analyses were performed by ANOVA and one sample test.

A reduction in HHI levels was observed in treated *vs* untreated rats ($p<0.05$), but no significant difference was detected either amongst T_4- or saline-treated animals ($p>0.05$). HHI levels were lower in 4A-6A than 4B-6B groups ($p<0.05$). SCG administration increased HHI levels in 7A *vs* 5A ($p<0.01$), but had no significant effect in 7B *vs* 5B group ($p>0.05$). Moreover, SCG attenuated the hyperactive and irritable attitude manifested by T_4-treated rats.

The data showed that the HHI content was decreased upon chronic T_4-treatment and that this effect was counterbalanced by pre-administration of the MC-membrane stabilizer SCG. This observation implied a possible interaction of T_4 and MC-derived HHI in the rat, though any contribution of histaminergic neurons cannot be excluded.

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Histamine Transport in Neonatal and Adult Astrocytes

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Type 1 astrocytes express a large number of neurotransmitter transporters, which are located in the vicinity of synaptic terminals where such uptake plays an important homeostatic function. Histamine uptake has been shown to occur via other neurotransmitters´ transporters, including the Oct2 transporter, into neurons, as well as via the extraneuronal monoamine transporter into nonneuronal cells. Uptake via extraneuronal monoamine transporter is Na^+- but not Cl^--dependent.

In this study, we measured histamine uptake into type 1 astrocyte cultures prepared from both neonatal and adult rat cortex. Histamine uptake was present in both cell types after one week in culture and was found to be Na^+-dependent and ouabain-sensitive, but not Cl^--dependent. Na^+-dependence and ouabain sensitivity appeared after three weeks of culturing for neonatal astrocyte but at two weeks for adult astrocytes. The Km (62 and 41 μM respectively) and Vmax (25 and 23 nmol/mg protein/min, respectively) are comparable for cells from neonatal and adult cortex. In contrast, uptake into mixed glial culture (including microglia, O2A progenitors, type 2 astrocytes and oligodendrocytes), obtained when the initial cell culture was shaken overnight, showed no Na^+-dependence or ouabain sensitivity.

These results support the possibility that type 1 astrocytes play an important role in the regulating excess of histamine content in the extracellular space and that histamine is taken up by extraneuronal monoamine transporter.

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Histamine and IL-1β interactions in PKC-stimulated NGF secretion from glial cells

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OBJECTIVE: Histamine stimulates nerve growth factor (NGF) synthesis and secretion from different types of cells via the activation of histamine H1-receptor and stimulation of protein-kinase C (PKC) and MAP-kinase [1]. IL-1β also posses stimulatory effect on NGF production which involves the activation of distinct protein kinases or inhibition of phosphoprotein phosphatases. Among this it can generate PKC cascade in certain transcription processes and acts synergistically with PKC-activators phorbol esters in the stimulation of NGF synthesis and secretion [2]. In the present work we were interested therefore to determine possible interactions between histamine and IL-1β in this process.
METHODS: As experimental model, we used primary cultures of rat neonatal cortical astrocytes, prepared from the brain of Wistar rats [3]. The cells were treated with different concentrations of histamine and IL-1β in the presence or absence of PKC inhibitors for different time of incubation. Released NGF was determined in the culture medium by NGF-ELISA.
RESULTS: The results showed that the treatment of the cells with both histamine and IL-1β significantly increases NGF secretion in comparison to the secretion, reached by either histamine or IL-1β alone. The enhancement of NGF secretion is dose and time-dependent. It becomes significant at concentrations of 100 nM of histamine and 1 ng/ml IL-1β and increases after prolonged time if incubation (up to 48 h). The effect of histamine and IL-1β is additive and can be partially blocked by PKC inhibitor Gö6976.
CONCLUSIONS: Our data indicates an additive effect of histamine and IL-1β in the regulation of NGF secretion from glial cells which is comparable to interaction between phorbol esters and IL-1β, where PKC-mediated pathway is of importance.
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Histamine-immunoreactive neurons in the mouse and rat suprachiasmatic nucleus

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Among the well-established roles of the neurotransmitter histamine (HA), is that as a regulator of the sleep-wake cycle, which early on gained HA a reputation as a "waking substance". The tuberomammillary nucleus (TMN) of the posterior hypothalamus, which contains the sole source of neuronal histamine in the brain, is reciprocally connected to the suprachiasmatic nucleus (SCN) which, in turn, is best known as the pacemaker of circadian rhythms in mammals.

We report HA-immunoreactive (-ir) neurons in the mouse and rat SCN which neither display immunoreactivity (-iry) for the HA-synthesizing enzyme histidine decarboxylase (HDC) nor contain HDC mRNA. Further, HA-iry was absent in the SCN of HDC knock-out mice but present in appropriate controls, indicating that the observed HA-iry is HDC-dependent. Experiments with hypothalamic slice cultures and i.c.v. injection of HA suggest that the HA of the SCN neurons originates in the TMN and is transported from the TMN along histaminergic fibers known to densely innervate the SCN. These results could be indicative of the existence of a hitherto unknown uptake mechanism for HA into neurons.

Through HA-uptake and, putatively, re-release of the captured HA, these neurons could participate in the histamine-mediated effects on the circadian system in concert with direct histaminergic inputs from the TMN to the SCN. The dense innervation of the SCN by several neurotransmitter systems could provide a way for other systems to directly affect the HA uptake by and release from neuronal cell bodies in the SCN.

Phenotypic profiling of experimental murine melanoma tumors with transgenically manipulated HDC expression

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In the present study, the impact of acquired neoplastic L-histidine decarboxylase (HDC) expression, and its direct consequence, the release of histamine in the tumor environment, was assessed on melanoma tumor progression.
B16-F10 mouse melanoma cells were manipulated via stable transfection, and nine novel transgenic variants were generated in triplicates, constitutively expressing the full-length mouse sense HDC mRNA, a mock control, and an antisense HDC RNA segment, respectively. Establishing both primary skin tumors and lung metastases in C57BL/6 mice, the nine variants with different histamine-releasing capacities were subjected to a comprehensive comparative progression profiling in vivo. Using RNase Protection Assay for screening of the melanoma progression profile, and Western blotting for subsequent result-validation, we made an attempt to identify tumor progression markers affected by melanoma histamine secretion.
Our analyses revealed trends of markedly accelerated tumor growth ($p<0,001$), and increased metastatic colony-forming potential ($p=0,010$) along with rising levels of local histamine production. Investigation of 21 functionally clustered markers associated with tumor proliferation, angiogenesis, invasivity, metastasis formation, local or systemic immunomodulation, and histamine signaling identified 2 markers possibly affected by histamine secretion. According to our results, a strong positive correlation is present between tumor histamine production, and markers of motility and metastatic potential (rho-C, $p=0,002$), and local histamine signaling (H2 receptor, $p<0,001$). These observations suggest that histamine represents an important component of the molecular machinery inducing and directing melanoma progression, and that distinct molecular pathways can be coupled to the complex phenotypic changes induced by histamine signals in developing melanomas.

Altered H_1 and H_2 Receptor Binding in the Hippocampal Areas of Subjects with Major Psychoses

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Alterations of both H_1 and H_2 receptor binding have been reported in the brains of patients with schizophrenia and depression, but no systematic studies have been carried out on the hippocampal area, one of the important limbic regions, in the major psychoses. Autoradiography, using [^{3}H]-mepyramine as H_1 receptor radioligand or [^{125}I]-aminopotentidine as H_2 receptor radioligand, was carried out on 4 matched groups of postmortem brain sections obtained from 15 schizophrenic subjects, 15 bipolar subjects, 15 depressive subjects and 15 controls. The materials were obtained from the Stanley Foundation Brain Collection.
In the anterior hippocampal area, significantly lower H_1 receptor binding levels were seen in both CA1 region and subiculum of all schizophrenic subjects and the depressive subjects who were treated with tricyclic antidepressants (TCA), as compared to the controls. In addition, H_1 receptor binding levels in the subiculum of bipolar subjects who were treated with either atypical antipsychotics (ATA) or TCA was also significantly lower than those of the controls. Significantly lower H_2 receptor binding levels were found in CA1 regions of the untreated bipolar subjects, bipolar subjects treated with medication other than ATA or TCA, schizophrenic subjects treated with typical antipsychotics, and depressive subjects treated with TCA, as compared to the controls. Decreased H_2 receptor binding levels were also seen in dentate gyrus of the medicated depressive subjects and bipolar subjects treated with medication other than ATA or TCA.
The results suggest that the antipsychotic drugs affect H_1 and H_2 receptor ligand binding in the hippocampal area. The disturbance of both receptors in the medication-free subjects suggests that histamine signaling through these receptors may be altered in these psychoses.

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H4R is Implicated in Various Effects Exerted by Histamine on Mouse Dendritic Cells

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Histamine has an important role in dendritic cell differentiation and also regulates cytokine production by these cells. It is not clear through which type of histamine receptors are these effects performed. In the last decade murine dendritic cells (DCs) were found to express –beside histamine H1 and H2 receptors –the novel histamine receptor H4 (H4R). In our study we used H4R-deficient (H4RKO) mice to demonstrate that histamine H4R has a critical role in dendritic cell development.

We have found differences in dendritic cell subpopulations between wild type and H4RKO mice using flow cytometry. Compared to wild type animals, less CD8α+ and more CD4+ dendritic cells were found in H4RKO mice, which suggests that in H4RKO mice the lymphoid-myeloid ratio is shifted towards myeloid origin. Real time PCR studies of dendritic cells demonstrated, that DC-s from H4RKO mice produce less Interleukin-10 (IL-10) and GATA-3. In addition, an increased level of the histamine producing enzyme, L-histidine-decarboxylase (HDC) and H1R expression was detected.

Our results provide evidence that histamine contributes to dendritic cell functions through the newly discovered H4 receptor.

Differential Effects of Histamine-Mediated Signaling Processes in Normal and Malignant Human Mammary Cells.

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The role of histamine (HA) in the growth of normal and cancer cells has been extensively investigated. We have previously reported that in rat normal mammary gland HA behaves as a mediator of cell growth under the control of specific growth factors. In experimental mammary carcinomas HA becomes an autocrine growth factor capable of regulating cell proliferation via H_1R and H_2R, as one of the first steps responsible for the onset of malignant transformation.
The aim of the present work was to investigate the differential signal transduction pathways in response to HA in normal (HBL-100) and cancer (MDA-MB-231) cells derived from human mammary gland. For that purpose we performed: proliferation and differentiation assays and cell cycle analysis. We also evaluated the reactive oxygen species levels and the expression of proteins related to proliferation, differentiation and apoptosis by flow cytometry. HA content was assessed by immunostaining and fluorescence was determined by flow cytometry and confocal microscopy.
In MDA-MB-231 cells, HA treatment modulated proliferation in a dose-dependent manner. HA 10 μM via H_2R increased cAMP production and H_2O_2 levels, decreased endogenous HA content as well as BCL-2, $ERK_{1/2}$ and P38 proteins, produced cell cycle arrest in G2/M and induced the expression of differentiation markers with the subsequent suppression of cell proliferation. In addition, HA at doses below 100 nM activated PLC via H_1R, enhancing cell proliferation and reducing H_2O_2 levels.
In contrast, in HBL-100 cells, HA content was significantly lower while exogenous HA only decreased cell growth at doses below 100 nM. Both H_1R and H_2R were coupled to PLC activation and increased H_2O_2 levels.
This study indicates that HA differentially regulates proliferation of normal and cancer cells representing a promising target for the development of specific breast cancer therapies.

AUTHORS´ INDEX: