IS BRAIN HISTAMINE INVOLVED IN DEPRESSION?

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The European Histamine Research Society
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Meeting report of the European Histamine Research Society

G. Sturman

The 40th meeting of the European Histamine Research Society was the second time we have met in Russia; the previous meeting being in 1995 in Moscow. This year the meeting was at the kind invitation of Professor Roman Khanferyan of Kuban State Medical University, Krasnodar, Russia and took place between 11 and 14th May 2011 at the Neva International Hotel, Sochi. Sochi, the largest resort of the Russian Federation, is situated on the eastern side of the Black Sea and will host the XXII Olympic Winter Games in 2014. This was the most easterly meeting our society has ever held. This year there were fewer delegates attending but they represented 11 countries (most from the European countries, especially Russia but also from North and South America and Japan). Some of the regular EHRS attendees could not be there and they were missed but a big welcome was given to all the new visitors, who we hope will return to future meetings.

Most delegates arrived during the Wednesday. As usual the Council held their meeting late afternoon before the Welcome Session. Our actual meeting was held in the hotels nightclub which had some easy chairs and sofas in the back row; a first for our society's meetings! Roman welcomed everyone and including those people from the European Academy of Allergy and Clinical Immunology. There was a welcome speech by Alexander Chuchalin, on behalf of the Russian Ministry of Health. Then we listened to a very special lecture given by Professor Wilfred Lorenz (Germany), one of our Honorary Members to celebrate our special anniversary. His presentation was entitled 'EHRS—Birth of the Histamine Club'. At the end of this delightful talk, Wilfred was given a little present to thank him. Then we listened to the first plenary lecture given by Nikos Papadopoulos (Greece) on 'Viruses and Asthma'. Then we moved to the restaurant for our Welcome Reception. There was a three piece band, which played for us while we nibbled on a wonderful array of canapés and drank local wines and, of course, vodka. We were able to wander and sit outside in the delightful water garden. In the corner of this garden, a couple of barbeques cooked for us. Old friends were greeted again whilst new ones were made.

On the Thursday, we started the meeting with the Opening Ceremony. We started with an introduction from our host Roman Khanferyan. Then the Mayor of Sochi talked about the developments of Sochi for the next winter Olympic Games in 2014. This was followed by the Head of the Russian Society of Immunology welcoming us to Russia. Then our president, Anita Sydbom gave her welcoming talk, which centred round Russian folk customs, especially folk dances. She then outlined the programme of this meeting. She then presented the student bursaries; certificates and cheques (each for €500) to six student members. Pfizer sponsored 2, while the G.B.West Memorial Trust gave another. The El-Sayed Assem family sponsored 2 more and the remaining one was from our society. Anita introduced the second of our plenary lectures which was given by Marek Jutel (Poland) and was entitled 'H_1/H_2 receptors in allergic and immune response'. After this very interesting lecture, the first of the oral sessions on Histamine in Allergy began. Like last year, each oral session at this meeting was chaired by an experience scientist as well as a young EHRS member so that they could get experience of chairing oral sessions. Just before our coffee break we had a group photograph taken. Then we listened to an oral session on Histamine H_3 and H_4 receptors, followed by one on Histamine and Metabolism, and then on Histamine H_4 Receptor and Immune Response. During the afternoon, we were able to view all the four posters sessions. One of the posters was a special one produced by Madeleine Ennis, one of our honorary members, celebrating our 40 years as a society with lots of photos of our highlights and history. The poster committee as usual had a difficult task in identifying winning posters for the poster competition. Eventually first prize was given to M. Kurnik et al. from Krakow, Poland, second to T. Karcz et al., also from Krakow and third prize to the Mexican group (Angelica Osorio-Espinoza et al.). In the evening we were taken to the City Concert Hall in the centre of Sochi where we listened to the Black Sea Jazz Group (Sergei Jemlanykhin, Sergei Kokorin, Rodion Latyshev, Vladimir Gritzai and Irina Babicheva) together with Robert Anchipolovsky (Israel) on saxophone. Because of the very high increase in humidity, the planned band was unable to fly into Sochi airport which was closed for a total of 4 days. Thus we all marvelled at Roman's ability to get this excellent band at such short notice. Of course, Roman ended up playing the piano with these musicians.

Friday began with the G.B. West lecture which was given by Robin Thurmond (USA) and entitled 'The role of the histamine H_4 receptor in allergy and inflammation'. This was followed by an oral session on Histamine and CNS and then one on Histamine and Inflammatory Responses. The planned plenary lecture by Igor Guskin (Russia) had to be cancelled, again due to the weather conditions preventing him flying into Sochi. After lunch we had a coach excursion to the historical Stalin's dacha. Here we learnt about Stalin and aspects of his life, including his love of billiards! Whilst we were at the Zelenaya Roscha house, a Round Table session on the 'H_1 verses H_4 receptor—what is important in allergy?' in was held in the meeting room. Delegates representing different fields of histamine research and chaired by Madeleine Ennis (UK) produced a lively debate about this new receptor and its role(s) in the body. In the evening there was more music and vodka tasting.

We had an early start on Saturday and were taken by coach through the lush Russian countryside up into the Caucasian mountains. We stopped to admire the views and saw the building of the Winter Olympic centre. At the newly built hospital for the Olympic Games we listen to the final plenary lecture given by our Treasurer, Patrizio Blandina (Italy) on 'Is brain histamine involved in depression?' The meeting organisers were very grateful to Patrizio for undertaking this lecture at short notice as the planned lecturer from Canada had cancelled at the last minute. We had a delightful lunch of grilled trout at the largest trout farm in Europe before heading back to our hotel. This time we were in a different venue in the hotel and it was much lighter. However, we were only to discover that the electricity had been cut only a short time before we needed it. Eventually we were able to listen to our students give their presentations for the Young Investigators Award. Regrettably one had to withdraw at the last minute due to a family illness. It was another difficult task for the judges (Fred Pearce, Frank Ahrens and Elena Rivera) in differentiating between these presentations. In fact, they awarded joint winners to Tobias Birnkammer (Germany) and Tommas Ellender (UK) with the others all being highly commended. Then we held our General Assembly. At the start of this meeting the death of one of our dear Honorary Members, Walter Schnuack (Germany) was reported and we all reflected on his generosity in supplying histaminergic compounds to members of the society as well as his wonderful character. According to our statutes, a new treasurer had to be elected and this was Pertti Paula (Finland) and we thanked the outgoing one, Patrizio Blandini (Italy) for his excellent work over the last 9 years. Our meeting ended with its traditional excellent Farewell Dinner followed by our award ceremony. The certificates and prizes were given out, the Black Sea Jazz band played for us again and Roman was often on the piano. Then as usual we had our singing session, beginning with "Anita's Thank You Song" (sung to the tune of This Ole House) as thanks to Roman and his team for the excellent meeting, and then we sung our EHRS Anthem.

We all agreed that the Russian team, especially Roman, had worked wonders in keeping the meeting running smoothly. It was a meeting of some unexpected events but with excellent science. Our thanks were given to all the Russians. The next meeting will be held in Belfast, Northern Ireland (2–5 May, 2012) at the kind invitation of Madeleine Ennis of Queens University Belfast—please see http://www.qub.ac.uk/EHRS2012 for more information about this meeting. This will be a joint meeting with the COST Action BM0806 group.

Walter Schunack (1935–2011)

H. Stark

Our dear friend, scientific colleague, and Honorary Member of the European Histamine Research Society Walter Schunack died in Berlin, Germany, on April 6, 2011, at the age of 76 after a short ordeal with an unexpected illness.

Prof. Dr. rer. nat. Dr. med. Dr. h.c. Walter Schunack has been one of the shining lights in medicinal chemistry for the design and the synthesis of new ligands for histamine receptor subtypes. The compounds he developed are used worldwide for the characterization of the pharmacological effects of histamine and its receptor subtypes.

Walter Schunack studied pharmacy and medicine in Mainz, Germany, made his PhD and MD, and started with the synthesis of histamine derivatives which have been also tested by him in a new pharmacological set-up at that time. In 1971 he went to the Freie Universität Berlin, Germany, as full professor and faithfully stayed there despite some possibilities for a change. He was passionately involved in the teaching of numerous generations of students and the advanced education for pharmacists and physicians in Germany. During his time he established "clinical pharmacy" as a new field for pharmacists.

In his research work he has largely been influenced by the works of E.J. Ariëns and Sir James Black on the characterization of physiological and pathophysiological effects of chemical mediators. Taking histamine as his starting molecule he has been able to develop numerous novel ligands with high affinity, high selectivity, and different pharmacological behaviour. (*R*)-alpha-Methylhistamine has been one of his major breakthrough findings for the classical pharmacological characterization of the histamine H_3 receptor subtype. Prodrugs such as BP2.94 were taken in development into clinical phase II. He developed the histamine H_3 receptor antagonist, pitolisant in collaboration with J.-C. Schwartz, J.-M. Arrang, M. Garbarg, C. R. Ganellin, J.-S. Lin, and H. Stark, and this has reached a new medical entity for the therapy of narcolepsy and excessive daytime sleepiness in Parkinson patients (late clinical phase III). Unfortunately, he will not be able to follow further the progress of this compound. Walter Schunack has been a key player in the field of ligands for histamine receptors as he has also been involved in the development of histamine H_1 receptor agonists (e.g. histaprodifen), radio-labeled antagonists for the histamine H_2 receptor (e.g. [^{125}I]iodoaminopotentidine), several reference ligands for the H_3 receptor (e.g. ciproxyfan, [^{125}I]iodoproxyfan), and specially designed hybrid compounds. With the recent cloning of the human histamine H_3 receptor he designed one of the first series of the nowadays mainly used non-imidazole ligands as a robust pharmacophore element. Although his work in the histamine area is best known to the scientific community he also investigated other neurotransmitters and G proteins.

He has received many national and international distinctions which have recently been mentioned with the election as Honorary Member of the European Histamine Research Society 2007 in Florence [1]. Walter Schunack is also Honorary Member of the Polish Histamine Research Society (2006), received the Distinguished Service Cross 1. Class of the Federal Republic of Germany (2002) and a third doctoral degree *honoris causa* by Univerité René Descartes, Sorbonne V, Paris (1998).

As a researcher and empathic personality he has been an extraordinary talented chemist and teacher, emphasized the clarity of scientific results and the passion for research. He has left an enduring imprint on histamine research. We leave Walter Schunack with great sadness, with even greater admiration and affection, and with the sincerest of condolence to his beloved wife and his devoted family.

Reference

1. Black J, Leurs R, Stark H, Sturman G. Honorary Membership of the European Histamine Research Society (EHRS). Inflamm Res. 2008;57(Suppl 1):S03–4.

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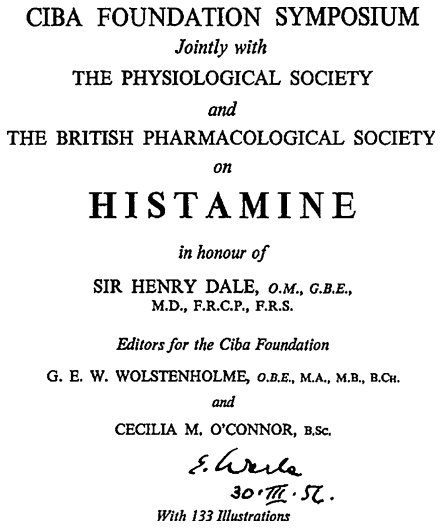
EHRS Anniversary Lecture—Birth of the Histamine Club

W. Lorenz

This year we celebrate the 40th anniversary of our society and I was asked to reflect on the start of the Histamine Club and who was involved. Unfortunately, there are only a few of us left who were members of the Histamine Club but as I am an Honorary Member of the EHRS I have been given the honour of talking about the birth of our society. I will attempt to do my very best—similar to the annual efforts on New Year's Eve of the butler James in the famous comedy "Dinner for One".

Probably the starting point was a Ciba Foundation Symposium on Histamine in 1955 (published in 1956) (Fig. 1). I received the Proceedings of this Symposium from Eugen Werle (my first tutor on the biochemistry of histamine) who participated in this meeting. It was dedicated to Sir Henry Dale and combined all the most influential histaminologists at that time. These included Feldberg, West, Gaddum, Riley, Sir Henry Dale, Rocha e Silva, Schild, Schayer, Code, Euler, Kahlson, Paton, Gregory, Zeller, Werle, Parrot, Halpern and Laborde as well as many others. It is interesting to note that at the end of the preface of the proceedings it reads—"Research on Histamine seems to be at a point at which important advances can be anticipated". Also, last sentence of the General Discussion mentions the existence of a *Histamine Club* whose aim was to bring scientists working on histamine together. Thus this Ciba Foundation brought about the birth of the Histamine Club. However the entry to this Club was made very prestigious as you needed two guarantors from the group of club members to be introduced. In 1969 I myself became a member of the Histamine Club and my guarantors were Czeslaw Maslinski (Poland) and my professor and tutor Eugen Werle (Germany). At the same meeting Jean-Charles Schwartz (France) became a member of this Club.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on
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in honour of
SIR HENRY DALE, *O.M., G.B.E.*, M.D., F.R.C.P., F.R.S.
Editors for the Ciba Foundation
G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.
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E. Werle
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With 133 Illustrations

Fig. 1 The Ciba Foundation Symposium on Histamine (1956)

The main advantage of the Histamine Club was to act as a forum to bring histaminologists together. In 1971 this became even more evident at the International Congress of Physiology in Munich and its Satellite Symposium on Histamine which was held in Lodz, Poland (Fig. 2).

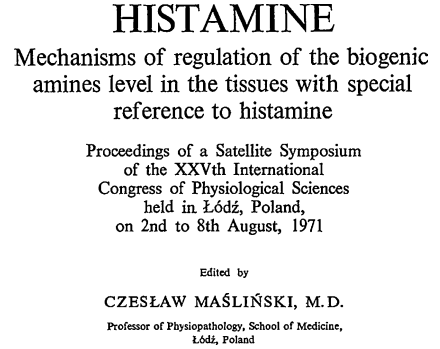
HISTAMINE
Mechanisms of regulation of the biogenic amines level in the tissues with special reference to histamine
Proceedings of a Satellite Symposium of the XXVth International Congress of Physiological Sciences held in Łódź, Poland, on 2nd to 8th August, 1971
Edited by
CZESŁAW MAŚLIŃSKI, M.D.
Professor of Physiopathology, School of Medicine, Łódź, Poland

Fig. 2 Proceedings from first unofficial meeting of the Histamine Club

At that time, Poland was part of the Eastern block countries and food was restricted. However, Czeslaw Maslinski found the classical Polish solution: vodka, sardines, sausages, bread and tomatoes—all from private sources! We also had time for an excursion on the River Wartha (Fig. 3). During that meeting I am sure that we never became completely sober but I still can recall the fantastic atmosphere. This was the first informal meeting of the Histamine Club which paved the way to our Histamine Society, and Jean-Louis Parrot was our General Secretary, a role which he retained until 1974.

Fig. 3 Our Histamine Club excursion on the River Wartha, Poland with Jean-Louis Parrot, our first General Secretary in the centre

Jean-Louis edited the annual Histamine Bulletin which contained a collection of abstracts from many papers on histamine in journals of various scientific societies, and managed the network of the Histamine Club. He was also Professor of Physiology at the Necker-Institute in Paris and a member of the Académie Française. In Lodz it was suggested that we have a General Assembly at our future meetings and after hefty debate this was accepted. This meeting was a considerable success—somewhat expected and somewhat sensational, but we were not able to recognize its significance immediately.

Jean-Charles Schwartz proposed Paris for the venue for the first official meeting of the Histamine Club in 1972 with Marburg for the following year (1973) and Copenhagen the next (1974). The Paris meeting ended with an excellent dinner in Jean-Charles' home hosted by his wife Kitty and the abstracts published in the French Journal of Pharmacology, Vol. 3 (1972). In 1974 Jean-Louis Parrot, who had done a great job as General Secretary handed over this role to Geoffrey West (UK). However, over the next few years it was felt necessary to change the established Club into a new scientific society. The Histamine Club became the European Histamine Research Society (EHRS) in 1979 but it still kept the 'family atmosphere' and encouraged members to help each other to solve histaminic problems. During Geoff's time the society drew up rules and regulations and also started to award poster prizes.

In 1972 there was a significant step in histamine research—the discovery of a histamine H_2 antagonist, burimamide by Black and co-workers. Jim Black, who previously was not known within the Histamine Club, made an immediate impact on all of us with his warm personality and excellent science. The clinical study of the new discovery using metiamide was rejected by The Lancet and so Jim Black published the randomized clinical trial results on metiamide in our newly founded journal Agents and Actions. This work led to the development of the clinically extremely successful cimetidine.

Black J. W., Duncan W. A. M., Emmett J. C. (1973) Metiamide – an orally active histamine H_2-receptor antagonist. *Agents and Actions 3*, 133–137.

Since then two more histamine receptors have been discovered; the H_3 and the H_4.

Fig. 4 Jim Black and Piero Mannaioni at one of our meetings

For 25 years, I was the editor for Agents and Actions, which was renamed in 1995 as Inflammation Research because several other international societies wished to also publish their meetings in the journal.

In 1988, Piero Mannaioni (Italy) (Fig. 4) became our General Secretary/President and now we are proud of our two lady EHRS presidents; Madeleine Ennis (UK), who had worked with Pearce and Lorenz (1999–2006) and Anita Sydbom (Sweden), who had trained under Uvnäs, the former Chair of the Nobel Committee and first President of the International Union of Pharmacology (IUPHAR) (2006–to date).

Last year we lost our dear Honorary Member, the Nobel Laureate Sir James Black (UK) whose achievements included being the senior author on the first paper on the H_2 antagonist (Nature 1972) and this year Walter Schunack (Germany) but the society does not stand still. The most remarkable feature of the EHRS is the strong friendship and close cooperation between participants

which has led to the development of European Research Groups resulting in important discoveries. For example, the development of reliable histamine assays and model systems (Uvnäs, Lorenz, Pearce) provided clear evidence for the clinical relevance of histamine and the synthesis of histamine agonists and antagonists (Schwartz, Gannelin, Schunack) provided the tools for our further research. The EHRS is still attracting more new members, especially young histaminologists, plus the science is excellent!

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40 years EHRS—science, fun and friends in Europe

M. Ennis

Unofficially the EHRS started with a meeting in Lodz, Poland in 1971, followed by the official establishment of the European Histamine Club in Paris in 1972. Geoff West (UK) became the Secretary General in 1974. In 1979, we became the European Histamine Research Society (EHRS) during our first ever meeting on a boat! This enabled one of our more forthright chairmen to declare that any speakers going over their allotted time would be thrown overboard! Piero Mannaioni (Italy) took over the reins in 1988, followed by me in 1999. However, I changed the title to President! Anita Sydbom (Sweden) became our President in 2006. She also performs a very interesting duty for the society, it started off as a one off in 1988 but now she has to write a special song for the meeting every year!

Meetings traditionally run for 3 days—well they actually start the night before and go on till breakfast on the final day, so really Wednesday to Sunday! We avoid parallel sessions as much as possible, so everybody stays to listen to all aspects of the subject. This can lead to some very interesting and thought provoking questions. We have an outing lasting an afternoon and evening and we often eat lunch en route! Essentially we spend all the time together—which results in many fruitful scientific collaborative projects as well as good friendships. The society has seen the growth of the Histamine field from 1 receptor to 4; resulting in many new areas for research. No longer just important in allergy but histamine plays a role in memory, obesity, ulcers, cancer etc.

Our idea of Europe is one that defies traditional boundaries so we have dedicated members from Japan, China, Korea, the Americas etc. It is a very friendly society, small enough so that you can speak to everybody and where young scientists are not afraid to go up to more senior colleagues and ask questions. Many even regard it as family! So as all families do, we miss those who are no longer with us, we benefited greatly from their wisdom. Long may the EHRS survive and thrive.

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Histamine H_3 and H_4 receptors

THE ROLE OF THE HISTAMINE H_4 RECEPTOR IN ALLERGY AND INFLAMMATION

R.L. Thurmond

Histamine is known to be one of the key mediators of allergic inflammatory conditions. However, evidence is now accumulating to suggest that histamine has a role in inflammation beyond that traditionally described for acute allergic symptoms. The discovery of a fourth histamine receptor (H_4R) 10 years ago and its expression on a number of immune/inflammatory cells has prompted the re-evaluation of the actions of histamine. The H_4 receptor mediates chemotaxis and cytokine release of mast cells, eosinophils, monocytes, dendritic cells and T cells. In addition, histamine released from mast cells or from other cell types can influence T cell polarization via activation of the H_4R. The receptor also mediates T cell activity in vivo and has a proinflammatory effect in models of the innate immune response like peritonitis or colitis models, but also in models of asthma and contact dermatitis, where it mainly effect T cell responses. These data suggest that the H_4 receptor is attractive target for the treatment of inflammation, allergy and asthma. The recent data on the novel functions of the H_4R have opened an exciting new chapter in the already impressive history of histamine research and should lead to new understanding and, hopefully, breakthrough therapies for the treatment of allergic disease.

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JNJ-39220675, A NOVEL SELECTIVE HISTAMINE H_3 RECEPTOR ANTAGONIST, REDUCES THE ABUSE-RELATED EFFECTS OF ALCOHOL IN RATS

R. Galici, A.H. Rezvani, L. Aluisio, B. Lord, E.D. Levin, I. Fraser, J. Boggs, N. Welty, J.R. Shoblock, S.T. Motley, M.A. Letavic, N.I. Carruthers, C. Dugovic, T.W. Lovenberg, P. Bonaventure

Recent studies suggest that brain histamine levels and signalling via H_3 receptors play an important role in modulation of alcohol stimulation and reward in rodents.

The present study characterized the effects of a novel, selective and brain penetrant H_3 receptor antagonist (JNJ-39220675) on the reinforcing effects of alcohol in rats.

The effect of JNJ-39220675 on alcohol intake and alcohol relapse-like behaviour was evaluated in selectively-bred alcohol-preferring rats using the standard two-bottle choice method. The compound was also tested on operant alcohol self administration in non-dependent rats and on alcohol-induced ataxia using the rotarod apparatus. In addition, alcohol-induced dopamine release in the nucleus accumbens was tested in freely moving rats.

Subcutaneous administration of the selective H_3 receptor antagonist dose-dependently reduced both alcohol intake and preference in alcohol preferring rats. JNJ-39220675 also reduced alcohol preference in the same strain of rats following a 3-day alcohol deprivation. The compound significantly and dose-dependently reduced alcohol self-administration without changing saccharin self-administration in alcohol non-dependent rats. Furthermore, the compound did not change the ataxic effects of alcohol, alcohol elimination rate nor alcohol-induced dopamine release in nucleus accumbens.

These results indicate that blockade of H_3 receptor should be considered as a new attractive mechanism for the treatment of alcoholism.

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MODULATION BY HISTAMINE H_3 RECEPTORS OF GLUTAMATERGIC TRANSMISSION IN RAT GLOBUS PALLIDUS

A. Osorio-Espinoza, A. Alatorre, J. Ramos-Jiménez, B. Garduño-Torres, M. García-Ramírez, E. Querejeta, J.-A. Arias-Montaño

The globus pallidus (GP), involved in the control of motor behavior, expresses high levels of histamine H_3 receptors (H_3Rs) and the virtual absence of the corresponding mRNA strongly suggests they are located on nerve terminals as auto- and hetero-receptors. The main synaptic afferents to the GP originate in the striatum (GABAergic), substantia nigra pars compacta (dopaminergic), subthalamic nucleus and cerebral cortex (glutamatergic). Here we studied the effect of the activation of rat pallidal H_3Rs on depolarization-evoked neurotransmitter release from slices, neuronal firing rate in vivo and turning behavior. Perfusion of GP slices with the selective H_3R agonist immepip had no effect on [^{3}H]-GABA or [^{3}H]-dopamine release evoked by high (20 mM) K^+, but significantly reduced [^{3}H]-D-aspartate release ($-44.8 \pm 2.6\%$, $n = 6$, and $-63.7 \pm 6.2\%$, $n = 3$, at 30 and 100 nM, respectively; $P < 0.05$, ANOVA and Student–Newman–Keuls test). The effect of 30 nM immepip was blocked by 10 μM of the selective H_3R antagonist A-331440 ($n = 5$). Intra-pallidal injection of immepip (0.1 μl, 100 μM) decreased spontaneous

neuronal firing rate in anaesthetized rats (peak inhibition 68.8 ± 10.3%, n = 13, $P < 0.05$) and this effect was reversed in a partial and transitory manner by A-331440 (0.1 μl, 1 mM, n = 4). In free-moving rats the intra-pallidal infusion of immepip (0.5 μl; 10, 50 and 100 μM, n = 7–9) induced dose-related ipsilateral turning following systemic apomorphine (0.5 mg/kg, s.c.). Turning behavior induced by local immepip (0.5 μl, 50 μM) and systemic apomorphine was partially prevented by local injection of the antagonist A-331440 (0.5 μl, 1 mM, n = 8) and was not additive to the turning evoked by the intra-pallidal injection of antagonists at ionotropic glutamate receptors (0.5 μl, 1 mM each of AP-5 and CNQX, n = 5–8, $P > 0.05$). These results indicate that pre-synaptic H_3Rs modulate glutamatergic transmission in rat globus pallidus and thus participate in the control of movement by basal ganglia.

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EFFECT OF HISTAMINE H_3 RECEPTOR ACTIVATION ON NEUROTRANSMITTER RELEASE FROM SLICES OF THE RAT OLFACTORY BULB

G. Aquino-Miranda, A. Osorio-Espinoza, J. Escamilla, J.-A. Arias-Montaño

The olfactory bulb is the first relay station of the central olfactory system in the mammalian brain. The rat olfactory bulb (rOB) possesses moderate histaminergic innervation but a very high density of histamine H_3 receptors (H_3Rs). H_3Rs are primarily hetero-receptors that control pre-synaptically the release of several neurotransmitters. Here we studied the effect of H_3R activation on the depolarization-evoked release of labeled neurotransmitters ([^{3}H]-GABA, [^{3}H]-D-aspartate, [^{3}H]-noradrenaline and [^{3}H]-dopamine) from rOB slices. The presence of pre-synaptic H_3Rs was confirmed by the specific binding of *N*-α-[methyl-^{3}H]histamine ([^{3}H]-NMHA) to membranes from rOB synaptosomes (maximum binding, B_{max}, 106 ± 19 fmol/mg protein and dissociation constant, K_d, 0.68 ± 0.11 nM, n = 4). [^{3}H]-NMHA binding was inhibited by the H_3R agonists immepip (p*K*i 9.49 ± 0.13) and *R*-α-methylhistamine (RAMH, p*K*i 9.10 ± 0.04) as well as by the H_3R antagonist clobenpropit (p*K*i 8.83 ± 0.07; n = 3). Perfusion of labeled rOB slices with the selective H_3R agonist RAMH (1 μM) had no effect on the release of [^{3}H]-GABA, [^{3}H]-D-aspartate or [^{3}H]-dopamine evoked by depolarization with high (20 mM) K^+, but reduced [^{3}H]-noradrenaline release in a modest although significant manner (83.1 ± 2.1% of control release, n = 3, $P < 0.05$, ANOVA and Tukey test). The inhibitory effect of RAMH (1 μM) on K^+-evoked [^{3}H]-noradrenaline release was blocked by the selective H_3R antagonist/inverse agonist clobenpropit (5 μM; n = 3). When tested alone clobenpropit and a second H_3R antagonist/inverse agonist, ciproxifan (both at 1 μM), increased K^+-induced [^{3}H]-noradrenaline release in a modest but significant manner (121.9 ± 5.9 and 117.0 ± 3.2% of K^+ alone, respectively, n = 3–4, $P < 0.05$). These results indicate that: (1) pre-synaptic H_3Rs do not regulate GABA, glutamate or dopamine release in rOB, (2) noradrenaline release in rOB is modulated by pre-synaptic H_3Rs with constitutive activity.

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THE ROLE OF THE HISTAMINE H_4 RECEPTOR ON HUMAN INVARIANT NATURAL KILLER T CELL SUBTYPE

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A functional role of the histamine H_4 receptor (H_4R) on the three main types of CD4+ T cells—Th1, Th2 and Th17 cells—has been described recently. Invariant natural killer T cells (iNKT cells) constitute the majority of unconventional or innate like T cells in the skin. Most recently iNKT cells were detected among infiltrating T lymphocytes in the lesional skin of atopic eczema and other inflammatory skin diseases in which they are exposed to histamine, raising the question whether histamine can regulate iNKT cell function.

Human iNKT cells are typified by a conserved invariant Vα24Jα18 T cell receptor chain paired with a Vβ11 chain and by their ability to recognize bacterial glycolipids and rapid production of several cytokines (e.g. IL-4 and IFN-γ). To investigate the influence of histamine especially via the H_4R on this rare subtype of T lymphocytes we first enriched these cells. To do this, we expanded iNKT cells in PBMCs using the glycolipid αGalactocylceramide and isolated the Vα24Jα18+ and CD3+ cells by fluorescence-activated cell sorting. The expression of the H_4R was evaluated by real-time PCR and by flow cytometry.

We could show for the first time the expression of the H_4R at the mRNA and protein level on human iNKT cells. To evaluate if the stimulation of human iNKT cells with selective H_4R agonists triggers the secretion of multiple

cytokines and cytotoxic molecules, we applied real-time PCR, flow cytometry and enzyme linked immunosorbent assay. We found no evidence for IL-4 and IFN-γ regulation in response to histamine and H_4R agonists. This is in contrast to recent published data on murine iNKT cells. Preliminary results show a possible effect of histamine via H4R on IL-22 regulation which requires further experiments.

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EFFECT OF HISTAMINE ON HUMAN NEUTROPHILS

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Recent data have shown that the histamine H_4 receptor (H_4R) is involved in chemotaxis of inflammatory cells such as eosinophils and neutrophils in asthmatic animal models. During episodes of allergic inflammation, chemoattractants guide neutrophils to the airways by switching $\beta 2$ integrins from a low affinity to a high affinity ligand binding conformation, thus allowing these adhesion receptors to bind endothelial ligands. This is a prerequisite for firm adhesion to the endothelium, migration to the site of allergic inflammation, and activation of $\beta 2$ integrin-dependent inflammatory functions (respiratory burst, degranulation). The aim of this study was to examine the effects of histamine on $\beta 2$ integrin function and neutrophil degranulation.

By flow cytometry, we showed that in resting neutrophils from healthy controls, around 1% of the cells express $\beta 2$ integrins in a high affinity ligand binding conformation. This percentage raised up to 55% in cells that had been stimulated with fMLP (0.1 mM), a potent chemoattractant, and up to 13.5% in neutrophils stimulated with histamine (10^{-7}–10^{-6} M). Surprisingly, despite the fact that histamine induced activation of $\beta 2$ integrins, the vasoamine blocked $\beta 2$ integrin-dependent degranulation. Indeed, incubation of human neutrophils with histamine (10^{-8}–10^{-6} M) blunted the release of lactoferrin (a marker of specific granules) in the extracellular milieu, induced by fMLP, or engagement of $\beta 2$ integrins. We also found, by flow cytometry, that human peripheral neutrophils, in addition to H_1R and H_2R, express also the H_4R.

We concluded that histamine may control $\beta 2$ integrin-dependent migration of human neutrophils via the H_4R whereas the vasoamine may control, in an inhibitory manner, inflammatory functions of neutrophils (degranulation) via H_1R or/and H_2R. This could be important in diseases such as severe neutrophilic asthma and COPD.

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PREVENTION OF BLEOMYCIN-INDUCED PULMONARY FIBROSIS BY A SELECTIVE HISTAMINE H_4R ANTAGONIST

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Pulmonary fibrosis is a progressive and lethal lung disease characterized by lung inflammation and abnormal remodeling of lung parenchyma. At the moment no cure exists for this disease. The histamine H_4R, widely expressed on cell of immune origin, plays an important role in inflammatory processes. We previously demonstrated that JNJ7777120 (JNJ), a H_4R antagonist, decreases the inflammatory response in animal models of asthma and carrageenan-induced pleurisy.

The aim of this study was to investigate the anti-inflammatory and anti-fibrotic effects of the histamine H_4R antagonist JNJ in a mouse model of bleomycin-induced pulmonary fibrosis. We also compared the effects of JNJ with those of naproxen, a classical NSAID. The bleomycin-induced mice were treated with vehicle or JNJ (total dose 40 mg/kg bw) or equimolar naproxen, released by micro-osmotic pumps. Airway resistance to inflation, an index of lung stiffness, was assayed and lung tissue was processed to evaluate inflammation and fibrosis biochemically and histologically.

JNJ exerted an anti-inflammatory effect, as shown by a significant decrease in the levels of PGE_2, myeloperoxidase, an index of leukocyte infiltration, and thiobarbituric acid reactive substances, markers of oxidative stress. JNJ administration also reduced the relative number of goblet cells and the thickness of smooth muscle layer, two key parameters of inflammation-induced adverse bronchial remodeling. Moreover, treatment with JNJ resulted in a strong inhibition of lung fibrosis, as shown by the reduction of the tissue levels of the pro-fibrotic cytokine TGF-β and of collagen; this was accompanied by a decrease in airway resistance to inflation. No significant differences were observed between the JNJ and naproxen treatment. Our results indicated that JNJ exerted an anti-inflammatory and anti-fibrotic activity and may offer a new therapeutic option for the treatment of pulmonary fibrosis.

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THE ROLE OF HISTAMINE RECEPTOR H_3 IN PANCREATIC β-CELLS

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H_3 receptor (H_3R), a G-protein coupled receptor (GPCR), is coupled to inhibitory G_i protein and exerts effects on various biological functions including sleep-awake cycle and cognition. A number of evidences have showed that H_3 receptor modulate hypothalamic neural activities leading to reduction of food intake and body weight. H_3R in central nervous system (CNS) has become one of the new therapeutic targets of obesity. There is, however, no information related to the role of H_3R in peripheral metabolic organs such as pancreatic β-cells, which are also involved in energy metabolism as well as CNS. Several GPCRs expressed in pancreatic β-cells such as glucagon-like peptide-1 receptor and α2A adrenergic receptor play important roles in insulin secretion and β-cell proliferation, whereas the expression and function of H_3R in pancreatic β-cells remain to be elucidated.

First, we found that H_3R was predominantly expressed among histamine receptors in mouse pancreatic β-cells and MIN6 cells, a cell line derived from mouse pancreatic β-cells. Next, we examined the effect of H_3R on insulin secretion from MIN6 cells using imetit, an H_3R agonist. Imetit remarkably inhibited glucose-induced insulin secretion in a dose-dependent manner, while basal insulin secretion with imetit was preserved. In addition, we investigated the effect of imetit on the proliferation of MIN6 cells and found imetit attenuated BrdU incorporation into MIN6 cells after insulin stimulation, suggesting H_3R decreases β-cell proliferation.

In this study, we have demonstrated for the first time that H_3R was expressed in mouse pancreatic β-cells and H_3R activation with imetit significantly inhibited insulin secretion and β-cell proliferation. These results indicate that H_3R may play an essential role in β-cell functions and be an eligible therapeutic target of diabetes in obese patients.

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ANTIHISTAMINE DRUG, DIMEBON, WITH MULTIPLE MECHANISMS OF ACTIONS

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Synthesized in Russia over 40 years ago and used clinically as an effective anti-allergic drug from 1983, is the drug Dimebon (9-/2-(2-methylpyridyl-5) ethyl/3,6-dimethyl-1, 2, 3, 4-tetrahydro-gamma-carboline dihydrochloride). In 2008, 'The Lancet' reported on the efficacy of Dimebon in patients with Alzheimer's disease with Dimebon. There was a significant improvement in Alzheimer's disease compared to placebo (mean drug-placebo difference -4.0 [95% CI -5.73 to -2.28]; $p < 0.0001$). Besides antihistamine activity, Dimebon has many other mechanisms of actions; blocking the action of neurotoxic β-amyloid proteins, inhibiting L-type Ca^{2+} channels, and modulating action of AMPA (a-amino-3-hydroxy-5-methyl-4-isoxasol-propionic acid) and NMDA (*N*-methyl-D-aspartate) glutamate receptor subtypes. Dimebon also blocks other receptors, such as α-adrenergic receptor and several serotonin receptors (5-HT_{2C}, 5-HT_{5A} and 5-HT_6). We have shown in experiments on guinea-pigs sensitized with ragweed pollen that Dimebon exerts marked anti-anaphylactic activity which compares with that of another Russian H_1 antagonist, Phencarol. When administered in minimal effective doses, Dimebon is less active than ketotifen, but in doses of 2.5 and 5 mg/kg it is more effective. Marked anti-anaphylactic activity of Dimebon is due to its antihistamine and antiserotonin actions. When administered intragastrically, s.c. and i.v., Dimebon was less toxic than Phencarol (by 1.28, 1.78 and 1.43-fold, respectively) and Ketotifen (by 1.78, 1.68 and 5-fold, respectively). In the rat Dimebon decreases the histamine-releasing activity of dextran T-70. In a series of experiments using cultivated PBMC from healthy donors in the presence of Dimebon, the H_3/H_4 antagonist imoproxifan was more effective in the modulating IgE response than other drugs. We are proposing that the antiallergic and CNS effects of Dimebon may be due to the antagonism of H_3/H_4 subtypes of histamine receptor.

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Histaminergic mechanisms in the CNS

IS BRAIN HISTAMINE INVOLVED IN DEPRESSION?

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Mice unable to synthesize histamine, due to targeted disruption of histidine decarboxylase (HDC) gene or i.c.v. injection of alpha-fluoromethylhistidine (α-FMH, 5 μg), a suicide inhibitor of HDC, were used to test roles for histamine in mediating behavioural changes elicited by different classes of antidepressants, selective serotonin reuptake inhibitors (SSRI) such as citalopram and paroxetine or selective noradrenaline reuptake inhibitors (SNRI), such as reboxetine. The tail suspension test (TST) which is a widely used paradigm for assessing antidepressant activity was used. Both classes of antidepressants reduced immobility in controls [wild type (WT) mice for HDC-KO mice, and saline-injected CD-1 mice for α-FMH-treated CD-1 mice (n = 11–18)]. HDC-KO mice as well as α-FMH-treated CD-1 mice failed to respond to acute injections of citalopram (10 mg/kg, i.p.) or paroxetine (10 mg/kg, i.p.) with a reduction of immobility. Conversely, reboxetine (5 mg/kg, i.p.) reduced immobility in both WT and HDC-KO mice ($p < 0.0001$). Since the histaminergic tuberomammillary nucleus receives 5-HT inputs from the raphé, we investigated whether SSRIs affect histamine release. CD-1 male mice (~30 g) were implanted with a microdialysis probe in the prefrontal cortex. After 48 h recovery, the probe was perfused with Ringer's solution (1 μl/min) and dialysates were collected every 30 min. Histamine was detected by HPLC-fluorometric detection. Spontaneous histamine release was 0.058 ± 0.006 pmol/30 min (n = 6). Citalopram (10 mg/kg; i.p.) increased HA up to more than 50% of basal value. 5-HT transmission appears intact in HDC-KO mice, since local infusion of citalopram (50 μM) into the hippocampus of HDC-KO mice increased by more than 100% the basal release of 5-HT, as measured by microdialysis. These data show that the central histamine system plays an important role in mediating acute behavioural and neurochemical actions of citalopram.

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CORRELATION OF PSYCHOTIC SYMPTOMS WITH CHANGES IN GLOBUS PALLIDUS H_3 RECEPTORS IN MAJOR HUMAN DEMENTIAS

N.L. Lethbridge, P.L. Chazot

Alzheimer's disease (AD) and Dementia with Lewy bodies (DLB) represent the two most common human dementias, each which display variable degrees of psychosis symptoms, which are difficult to treat with current therapies. In this study, we determined the levels of H_3 histamine receptors (H_3Rs), using [^{3}H] GSK189254 autoradiography and post-mortem tissue from a cohort of validated AD and DLB cases (Newcastle Brain bank, UK).

Whereas many other brain structures remained unchanged, the globus pallidus H_3Rs displayed a range of changes in AD and DLB cases in comparison to control cases. Differential bidirectional changes in [^{3}H] GSK189254 binding levels were observed in male AD (no change), DLB (decrease) and control cases (increase to level seen in AD cases); in contrast female AD and DLB cases remained constant (n = 3–4 individual cases).

These cases were further analysed to assess whether presence of delusions and visual hallucinations correlated with changes in globus pallidus H_3Rs. Overall, the psychotic positive symptoms assessed (delusions and visual hallucinations) did not correlate for a range of cortical and basal ganglia structures, with the exception of the globus pallidus. DLB cases with moderate to high delusion and visual hallucination scores displayed approx. 40% higher [^{3}H] GSK189254 binding levels (n = 6–7 individual cases), in comparison to cases lacking such psychotic symptoms. There was a trend for a similar correlation for AD cases. The volume of the human globus pallidus has been previously positively correlated with the severity of global psychotic symptoms, as measured by both the Scale for the Assessment of Negative Symptoms and Positive Symptoms, which may account for this apparent increase in H_3 receptors.

This provides further evidence for the potential use of H_3R antagonists in treating the psychotic symptoms in schizophrenia and, perhaps, Lewy body dementias.

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THE HISTAMINE H_3 RECEPTOR HAS A LIMITED ROLE TO PLAY IN ANXIETY BEHAVIOUR

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Our new developed and patented open space test system for measuring anxiety responses has been validated with different strains of mice (e.g. Balb/c high anxiety and CD-1 low anxiety strain). It has been shown to be sensitive to a standard clinical anxiolytic agent. This represents the first behavioural test which can discriminate anxiety and hyperactivity responses, and can be used in multiple sessions for assessment of chronic treatments. In this study, 2 month old Balb/c mice were divided into four groups

(8 animals per group) and received either saline, or a H_3R antagonist GSK334429B at 0.1, 0.3 or 1 mg/kg i.p. (supplied by Dr AD Medhurst, GSK) and then exposed to the open space test (elevated configuration). This behavioural paradigm simultaneously examined emotional responses to novelty and open spaces and motor activity over 5 daily 12 min. sessions. Motor activity and anxiety measures for a mouse on the open space behavioural paradigm consisted of the number of crossings in the surface of the platform, latency of the first entry, number of entries and duration of entries onto the stands, respectively. An increase in number of crossings onto the stands, increased time spent on stands, and a reduction in the latency of first entry to the stand simultaneously would reflect a lower level of anxiety (an anxiolytic agent). There were no significant differences between any of the 4 groups indicating that GSK334429B is neither anxiolytic nor anxiogenic. Majority of results were statistically insignificant, but there was significance between sessions. This was to be expected, as the more exposed the mice are to the test they would begin to overcome the anxiety of the novelty and open space environment.

This study provided new evidence that that the H_3 histamine receptor, while it may be involved in fear-avoidance responses, it is not implicated in anxiety responses. This concurs with other unpublished studies from our laboratory using the 3D maze open space anxiety test, together with both selective H_3 agonist and antagonist drugs.

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FORMAL IDENTIFICATION OF HUMAN NATIVE H_3 RECEPTOR (H_3R) ISOFORM PROTEINS AND POTENTIAL DIFFERENTIAL CHANGES IN H_3R ISOFORMS IN MAJOR LEWY BODY DISEASES

N.L. Lethbridge, P. Chazot

We have developed and characterised a selection of novel isoform selective human H_3R probes: anti-H_3R PAN antibody, anti-$hH_3R_{445/453}$ antibody, anti-$hH_3R_{365/445}$, anti-hH_3R_{329} antibody and anti-hH_3R_{200} antibodies. Selectivities were confirmed utilising respective cDNAs expressed in HEK 293 cells and probed by comparative quantitative immunoblotting. Herein, these probes were used to identify for the first time, human H_3R isoform proteins expressed in vivo in human putamen, and to determine using quantitative immunoblotting ($n = 3$–4 individual samples per group), whether there were any changes in expression of H_3R isoforms in human Lewy body disease.

There were no significant difference in immunoreactivities between the two disease states and control samples, indicating that the full length $hH_3R_{445/453}$ expression is unaltered in Parkinsons Disease (PD) and Dementia with Lewy Bodies (DLB) compared with control cases. There were no significant differences in immunoreactive signals detected with the anti-$hH_3R_{365/445}$ antibody between the two disease states, with the exception of the putative H_3R 365 isoform, where there was a significantly stronger immunoreactivity signal in DLB, but not in PD, versus control cases. In contrast, there was a significant increase in anti-hH_3R_{329} immunoreactivity in the PD cases compared to both control and DLB cases.

These results suggest that H_3R 365 and H_3R 329 isoform expression maybe up-regulated and down-regulated in DLB and PD, respectively, while H_3R 445 isoform remains largely unchanged in both Lewy body dementias.

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EFFECTS OF MONOAMINE SYSTEM DIRECTED MULTITARGET DRUG ON RAT BRAIN AMINE TRANSMITTERS AND ON COGNITIVE FUNCTIONS IN RAT MODEL OF VASCULAR DEMENTIA

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Deficits in amine neurotransmitters are seen in neurodegenerative diseases and currently the idea is to use multitarget drug treatment. ASS234, a novel compound in vitro suppressing MAOA and B, and AChE/BChE activities, was examined for its effect on deteriorated memory in Wistar rats with post bilateral common carotid artery occlusion (BCCAO). The drug was given daily (5 mg/kg, s.c.) for 5 days to intact or 1 day post BCCAO surgery. Working and reference memory was evaluated using hole-board tests; before and 7 and 12 days after BCCAO. Enzyme activities MAOs and histamine *N*-methyltransferase were measured by radioassays and acetylcholinesterase with an AChE fluorescent kit. Amine concentrations analysed with RIA kits (5HT, DA, NA), radioenzymatically or fluorimetrically (histamine) and by GC/MS (t-methylhistamine). In the brain, ASS234 treatment resulted in 60% inhibition of MAO B activity and undetectable MAO A. However, 5HT concentration increased >3 times, dopamine and

noradrenaline > twice. The histamine system was less affected: histamine and t-methylhistamine levels increased by ~30% and there was a small decrease in HMT activity (40 ± 0.7 vs. 44 ± 1.1 pmol/min/mg protein, $p < 0.05$); the last could be a direct effect of ASS234 on HMT or substrate inhibition (methylhistamine). AChE activity as measured in striatum, decreased insignificantly. However, AChE has a very high turnover rate and the drug was only given once a day. Food and water intake and urine production were all decreased. This could be facilitated by brain 5HT increase but histamine involvement cannot be excluded. Interestingly the ASS234 treated BCCAO rats showed less negative effects of cerebral hypoperfusion (BCCAO) on behavioural memory parameters. Thus the amine system, due to complicated interplays, should be targeted as a whole entity.

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DIFFERENTIAL CONTROL OF STRIATAL INHIBITION BY HISTAMINE

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The striatum receives modulatory input from the hypothalamic histaminergic system. Histaminergic neurons exhibit a diurnal rhythm in their activity, being active during day and relatively silent at night. Thus the striatum is likely to experience a relatively high concentration of histamine (H) during the day. This, combined with the high expression of H receptors in the striatum, suggests that H might have a strong regulatory role in striatal function.

We investigated the role of H in the modulation of the main excitatory and inhibitory inputs to the principal neuron of the striatum, the GABAergic medium spiny projection neurons (MSNs). We performed whole-cell patch-clamp recordings of single MSNs, pairs of connected MSNs and pairs of connected interneurons and MSNs. We investigated the effect of bath-applied H (10 μM) in conjunction with selective H receptor antagonists on the excitatory responses evoked by cortical or thalamic stimulation or inhibitory responses evoked by local stimulation or unitary inhibitory responses between pairs of neurons.

We found that both cortical and thalamic inputs to MSNs were negatively modulated by bath applied H, which was prevented by co-application of the H_3 receptor antagonist, thioperamide (10 μM). Similarly, inhibitory inputs evoked by local stimulation were negatively modulated by bath applied H. However, in paired recordings only the unitary inhibitory responses between pairs of MSNs were significantly reduced, whereas those between fast spiking interneurons and MSNs were unaffected.

Thus the histaminergic innervation of the striatum is involved in the negative regulation of both of the main excitatory inputs and the feed-back inhibitory input between MSNs, but does not affect the feed-forward inhibitory input from the fast spiking interneurons. Selective attenuation of feed-back inhibition by H will alter the striatal processing of excitatory inputs and by extension the expression of basal ganglia behaviour.

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SPECIFIC EFFECTS OF CENTRAL H_1 ANTAGONISM ON SENSORI-MOTOR PERFORMANCE AND BOLD RESPONSE

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Histamine plays a role in cognitive functioning and sensori-motor functioning in particular, comprising of many processes from visual input to motoric output. However, the histaminergic role in these processes is largely unknown. Recently, effects of H_1-antagonism on brain evoked potentials indicated that sensory visual processes are affected, but not response related processes. In addition, the brain may attempt to compensate for this impairment by speeding up response choice related processes. To establish the effects on visually related processes and test the 'compensation' hypothesis, we conducted a functional Magnetic Resonance Imaging (fMRI) study to assess brain activity changes related to increased visual and response choice task demands and interaction with H_1-antagonism.

Using a 3-way cross over design, 9 subjects (5 female) with a mean age of 20.6 years (SEM ±0.8) received single oral doses of dexchlorpheniramine 4 mg (D4), lorazepam 1 mg and placebo. Brain activity was measured in a 3T MRI scanner 2 h post dose when subjects performed a choice reaction time task. Subjects were required to respond with their left or right hand as indicated by the stimulus presented left or right side of the screen and using their index or middle finger as indicated by the identity of the stimulus. Task manipulations were visually intact or degraded stimuli and compatible or incompatible

responses, manipulating visual and response choice processing demands.

Degraded stimuli increased brain activity in primary visual areas, but incompatible responses did not activate a response choice network. D4 reduced brain activity following a degraded stimulus in nine areas, but had no expected effect in the visual cortex. D4 also did not interact with response choice demands. There was no evidence of compensatory activity in response choice related areas following degraded stimuli and D4, but compensation might have taken place in language, working memory and attention related areas.

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Immunological aspects of histamine

VIRUSES AND ASTHMA

N. Papadopoulos

Viral infections have been associated with the initiation, protection and exacerbation of asthma; interactions with other stimuli such as allergens have also been demonstrated. Among the different respiratory viruses, human rhinoviruses (RV) have been associated with the majority of asthma exacerbations and induction of asthma in childhood. RV are able to infect and replicate in the lower respiratory epithelium, where they induce a local inflammatory response. Nevertheless, the immune response to RV is impaired in atopic individuals with reduced IFN-gamma responses and defective co-stimulation. Defects in the interferon response are characteristic in atopic asthmatics leading to diminished viral clearance and possible perpetuation of inflammation. Epithelial cells, if placed in an atopic environment, fail to clear the virus and are susceptible to increased cytotoxicity. Simultaneous or subsequent exposure to allergen further increases inflammatory responses through an NF-kB mediated mechanism. RV infections are also able to induce production of remodeling-associated factors, such as VEGF and FGF-2, which further augmented in an atopic environment may result in virus-induced remodelling. In the clinical setting, atopic children with virus-induced asthma are much more likely to continue being hyperresponsive than non-atopic children.

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H_1/H_2 RECEPTORS IN ALLERGIC AND IMMUNE RESPONSES

M. Jutel

Histamine (H) has been shown to affect chronic inflammation and to regulate essential events in the immune response. These effects are due to the differential expression and regulation of 4 distinct receptors (H_1–H_4R). The discovery of reciprocal regulation of T cell activity by H_1 and H_2R activation, indicating H-cytokine cross-talk, plus characterization of the H_4R and its expression on many immune and inflammatory cells, has led to a re-evaluation of H actions. H is involved in the regulation of immune responses and haematopoiesis and affects many cells including macrophages, dendritic cells (DC), T and B lymphocytes and endothelial cells. H directly affects DC (both immature and mature) which express all 4 R. In the differentiation process of DC1 from monocytes, H_1R and H_3R act as positive stimulants which increase antigen-presentating capacity, proinflammatory cytokine production and Th1 priming activity. However, activation of H_2R acts as a suppressant for antigen-presentation by enhancing IL-10 production and inducing IL-10-producing T cells or Th2 cells. Differentiation of monocytes into macrophages also induces a dramatic down-regulation of H_2R, while differentiation into DC has no significant influence on H_2R expression. Over-expressed H_1R is functionally active and induces increased production of IL-8 in H-stimulated macrophages. H intervenes in the Th1, Th2, Treg cell balance and thus antibody formation. Differential patterns of HR expression on Th1 and Th2 cells determine reciprocal T cell responses after H stimulation. Th1 cells show a predominant, but not exclusive, expression of H_1R while Th2 cells show increased expression of H_2R. H enhances Th1-type responses via the H_1R, while both Th1- and Th2-type effects are negatively regulated by H_2R. Deletion of H_1R in mice causes suppression of IFN-α and dominant secretion of Th2 cytokines (IL-4 and IL-13) while H_2R deletion leads to upregulation of both Th1 and Th2 cytokines. Bphs, a non-major histocompatibility complex-related gene involved in susceptibility to many autoimmune diseases, has been linked to the H_1R in mice and knockout mice show delayed disease onset and decreased severity in models of experimental allergic encephalomyelitis. H stimulation also induces IL-10 secretion via H_2R. Increased IL-10 production in both DC and T cells may provide an important regulatory mechanism for the control of inflammatory functions. Various cytokines regulate the production of H and its R expression. IL-3 and GM-CSF enhance H release from basophils and SCF acts similarly on mast cells. IL-3 stimulation significantly increases H_3R expression on Th1 but not Th2 cells. H also interferes with induced peripheral tolerance. H induces IL1-10 production from DC and enhances the suppressive activity of TGF-α on T cells. All of these effects are via the H_2R which is highly expressed on Th2 cells and mediated through suppressed IL-4 and IL-13 production and T cell proliferation. This suggests that the H_2R may be essentially involved in peripheral tolerance or active suppression of inflammatory/immune responses. Future work on the role of H and other G-protein-coupled R in inflammation and the immune system is required.

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HISTAMINE H_1 AND H_4 RECEPTOR ANTAGONISTS DO NOT PREVENT ACUTE SKIN LESIONS IN A CANINE MODEL OF ATOPIC DERMATITIS

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Highly selective histamine H_4 receptor (H_4R) antagonists display anti-inflammatory and anti-pruritic in vivo efficacy in mice. Our objectives were to test H_4R antagonists for skin lesion prevention in a canine model of atopic dermatitis and to compare their efficacy to that of two H_1R antagonists that are used as add-on therapy to treat acute atopic lesions and pruritus in dogs.

In these blinded, placebo and active-controlled cross-over experiments, six Maltese-beagle atopic dogs sensitized to house dust mite antigen were treated topically with 1% solutions of the H_4R antagonists JNJ7777120 or JNJ28307474 (kindly provided by Johnson & Johnson, Pharmaceutical Research & Development, La Jolla, USA) or orally with JNJ28307474 at 15 mg/kg before and immediately after allergen challenge. Pretreatment with 0.015% triamcinolone acetonide solution served as a positive control. In a second trial, the H_1R antagonists, hydroxyzine pamoate (2 mg/kg twice daily) and cetirizine (0.5 mg/kg once daily) were administered orally before challenge. Twenty-four hours after challenge by epicutaneous application of house dust mite antigen, erythematous skin lesions were scored. Lesional scores after challenge were not significantly different after placebo, JNJ28307474 (orally or topically administered) and JNJ7777120 (topically administered). Hydroxyzine and cetirizine also did not reduce the median score of the placebo treatment, while triamcinolone acetonide prevented all dogs from developing any lesions. Increased concentrations of histamine, as determined by microdialysis, were detected in lesions only during the initiation stage of the process.

Thus, the administration of H_1R or H_4R antagonists did not prevent the development of acute atopic skin lesions in this canine atopic dermatitis model. However, the glucocorticoid positive control led to the expected prevention of lesions.

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CHANGES IN PLASMA HISTAMINE CONCENTRATION IN A DAIRY HERD AFTER CHANGING THE FLOORING FROM SLATTED CONCRETE TO SLATTED RUBBER MATS

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Lameness in dairy cows is a major economic and welfare issue. Histamine seems to play a crucial role in laminitis, a condition that impairs blood circulation in horn-producing tissue of the foot. Therefore, the aim of this study was to investigate the effect of changing the flooring in the alleys of a barn from slatted concrete to slatted rubber mats on hoof health, plasma histamine concentration, stress and the immune system in 44 loose-housed Brown Swiss dairy cows.

Cows were examined for hoof disorders of the hind limbs (haemorrhages, white line fissures, ulcers, heel horn erosion, and digital dermatitis). Evaluations were carried out when the cows were housed on a concrete slatted floor and after 4 and 10 months on soft flooring (slatted rubber mats, 29 mm thick). In 15 cows, 3 blood samples were drawn when the cows were still housed on concrete slats and another 6 samples were taken when the cows were housed on rubber slats. Histamine concentration and parameters for assessing the stress response (cortisol) and the immune system (immunoglobulin G) were measured in plasma by HPLC (histamine, cortisol) or by ELISA (immunoglobulin G).

Changing the floor from slatted concrete to slatted rubber mats significantly increased the score for white line fissures (after 10 months on rubber mats) while the other hoof disorders were not affected. Immunoglobulin G and cortisol concentration in the plasma increased when the cows were housed on rubber slats. The opposite applied to the plasma histamine concentration which was significantly higher when the cows were housed on concrete.

Thus covering slatted concrete flooring with slatted rubber mats affected hoof health only partially but significantly influenced blood parameters of inflammation (histamine), stress (cortisol), and the immune system (immunoglobulin G) in dairy cows.

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IMAGING HISTAMINE H1 RECEPTORS USING PET AND [^{11}C]DOXEPIN: FURTHER PROGRESS

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Histaminergic neurons are exclusively located in the posterior hypothalamus and project their fibers to almost all regions of the brain. We have now examined the functions of these neurons in humans using positron emission tomography (PET). We here summarize our recent work on [^{11}C]doxepin-PET studies, including gender differences in histamine H_1 receptors and the 'hangover effects' of nocturnally-administered sedating antihistamines.

We found that there are gender differences in the interstitial nuclei of the anterior hypothalamus. The nuclei of homosexual men are only one-half the size of those of heterosexual men. Previous studies demonstrated the sexual dimorphism of brain histamine and behaviour in animals. In accordance with animal studies, normal female subjects show significantly higher binding of [^{11}C]doxepin to H_1 receptors in the cerebral cortical areas than male healthy volunteers ($N = 12$). Gender differences should then be taken into consideration in studies of neuronal histamine.

Antihistamines are frequently used for the treatment of allergic diseases, common cold, cough, fever or motion sickness. Sedation is a well-recognized central side-effect of first-generation antihistamines and is caused by blockade of the histaminergic neuron system in the human brain. To avoid daytime sedation, antihistamines are often administered at night. However, the next-day residual sedative effect of antihistamines, the so-called 'hangover effect', has never been quantitatively evaluated by PET. Diphenhydramine (50 mg), a sedating antihistamine, retained predominant residual effects as demonstrated by its high central H_1 receptor occupancy (average 44.6%) even 12 h after taken orally ($N = 8$). Attention should thus be paid to a possible cognitive decline or impaired performance on the day after treatment with sedating antihistamines even when they are administrated at night.

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THE EFFICACY AND SAFETY OF TREATMENT OF ALLERGIC CHILDREN WITH NEW GENERATION OF ANTIHISTAMINES

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The new generation of histamine H_1 receptor antagonists have been shown to be very efficacious and safe. They are mainly recommended for the treatment of allergic rhinitis, urticaria and other allergic conditions. The aim of this study was to investigate parent and physician satisfaction with oral antihistamine treatment in children and to compare levocetirizine with other antihistamines. In an international Observational Survey in Children with Allergic Rhinitis (OSCAR), children (2–12 years old with a history of allergic conditions) were enrolled from 424 primary-care/specialist allergy clinics from Bulgaria, India, Portugal, Romania, Russia, South Korea and Spain. At the consultation, parents and physicians completed questionnaires evaluating their satisfaction with specific antihistamines currently used for management of the child's allergic condition, plus their intention for future use of that treatment. A total of 4581 patients were enrolled: 3048 (66.5%) had allergic rhinitis (55.9% persistent allergic rhinitis and 44.1% intermittent allergic rhinitis) and 663 (14.5%) had urticaria as primary conditions. Also, 2465 patients (53.8%) suffered from other allergic conditions, including allergic asthma, atopic dermatitis, food allergy and drug hypersensitivity. Levocetirizine and fexofenadine scored highest for efficacy, tolerability and global satisfaction, and for impact on the child's ability to function at school, quality of school activities and quality of sleep. Parent and physician satisfaction scores were in close agreement and demonstrated a significantly greater global satisfaction for the second-generation antihistamines. This study shows that second-generation antihistamines, particularly levocetirizine and fexofenadine, have superior risk:benefit ratios compared to first-generation drugs. Levocetirizine is thus one of the drugs of choice in the treatment of allergic diseases in children.

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INVOLVEMENT OF HISTAMINE RECEPTORS IN TOTAL AND ALLERGEN-SPECIFIC IgE SYNTHESIS

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Effects of histamine are mediated through different types of receptors, which are all G protein-coupled. It has been shown that histamine (H) is involved in IgE regulation. This study assesses the impact of H_1, H_2 and dual H_3/H_4 H receptor antagonists on IgE synthesis by peripheral blood mononuclear cells (PBMC) from healthy donors plus patients sensitized to ragweed pollen. Supernatants of 9-day cultures of peripheral mononuclear blood cells (PBMC) from healthy donors and ragweed sensitive patients were assayed for total and specific anti-ragweed IgE by the CAP FEIA method (Phadia) and ELISA.

H_1 (Loratadine) and H_2 (Cimetidine) antagonists as well as the H_3/H_4 antagonist, Imoproxifan (IMP) ($K_i = 0.26$ nM) were used as H receptor antagonists. PBMC from ragweed sensitive patients were also stimulated by ragweed allergen (Greer) with the 10 IU concentration of antigen E. Level of the synthesis of homocytotropic anti-ragweed specific IgE-Ab has been studied by passive cutaneous anaphylaxis in a mouse model. It was shown that H_1 and H_2 antagonists injected before immunization bidirectionally influenced IgE-specific Ab synthesis in mice. Simultaneous blockade of H_1 and H_2 receptors prior to injections of H_3/H_4 antagonist IMP modulates the impact of this antagonist on IgE-Ab synthesis. In vitro studies showed that simultaneous blockade of H_1 and H_2 receptors dose-dependently increased IgE synthesis by PBMC of healthy donors incubated with H_3 receptor agonist R-α-methyl-histamine (RMH). Similar results in total IgE synthesis was achieved in culture of PBMC of ragweed sensitive patients. IgE specific Ab synthesis modulated similarly total IgE synthesis when PBMC culture of ragweed sensitive patients were cultivated in presence of H_1, H_2 and H_3/H_4 histamine antagonists.

Thus, different types of H receptor antagonists modulate total IgE plus allergen specific IgE synthesis in healthy donors and ragweed-sensitive patients. Simultaneous blockade of H_1 and H_2 receptors influence the IgE-modulatory effects of an H_3/H_4 antagonist and H_3 agonist.

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DYNAMICS OF SERUM CONCENTRATION OF THE HISTAMINE, TNF-α AND IL-10 IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH INFLIXIMAB

S.P. Oransky, L.N. Eliseeva, J.V. Vasinova, R.A. Khanferyan

Here is no unique understanding of the effects of histamine and its possible influence on cytokine profile in autoimmune diseases, particularly in rheumatoid arthritis (RA). For a long time opinion existed that the prevalence of proinflammatory effects of histamine in RA was mediated through H_1 receptors. Later from experimental evidence, the ability of histamine, via the H_2 receptor, could switch Th1 lymphocytes to Th2 types with the increase in synthesis of anti-inflammatory cytokines.

The purpose of this study was to investigate the dynamics of serum histamine, TNF-α and IL-10 in patients with RA, treated with monoclonal antibodies against TNF-α (infliximab). 25 patients with active RA and a control group of 12 healthy volunteers were included in the clinical trial. Serum histamine and levels of TNF-α and IL-10 were measured before and after five intravenous infusions of infliximab.

It was shown that serum concentration of histamine in healthy donors and RA patients before treatment did not differ significantly with a level of 0.8 (0.2; 1.1) ng/ml in RA. The concentration of cytokines was significantly higher when compared with the control group: in RA patients the concentration of TNF-α was 178.1 (101; 243) and IL-10 302.5 (208.3; 510) pg/ml. After infliximab therapy there was a significant decrease in the concentration of TNF-α of up to 74.3 (59; 120) pg/ml ($p = 0.02$). Infliximab did not influence the serum concentration of IL-10. Histamine concentration after infliximab injection was slightly increased to 1.4 (1.2; 1.9) ng/ml ($p = 0.04$). Therefore it was shown that infliximab has the ability to reduce proinflammatory cytokine activation in RA patients and this effect was accompanied by an increase in histamine concentration.

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EFFICACY OF IMMUNOMODULATORY THERAPY IN CHILDREN WITH ATOPIC DERMATITIS

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It has been shown previously that the inclusion of the new Russian immunomodulatory drug Licopid (active ingredient is *N*-Acetylglucosaminyl-(β1 → 4)-*N*-acetylmuramyl-alanyl-D-isoglutamine) into the complex therapy of IgE-mediated atopic dermatitis (AD) increases the efficacy of the treatment. The purpose of this study was to examine the clinical effects of the therapy of 85 children (6–9 years) with IgE-mediated moderate AD accompanied with local infections. Patients without infections at the moment of observation were divided into 3 groups: group A (25 children) received only standard therapy (UT), group B (30 children) received UT in combination with Licopid at a total dose of 25 mg, and group C (30 children) received a combination of UT with Licopid at a higher dose of 50 mg. Clinical efficacy according to SCORAD parameters was analyzed before the treatment and 1, 2 and 6 months after the treatment. Analysis of results showed a decrease in the number of aggravations of AD in clinical group C, in comparison to a prevalence of aggravations in groups A (3.4-fold) and B (twofold). Moreover, there was a marked decrease in the number of aggravations of accompanying

diseases in group C. In total, these results suggest that the modified scheme of therapy with high doses of Licopid (50 mg in total) may be highly efficacious in the treatment of children with IgE-mediated moderate AD.

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DEVELOPMENT OF A TOPICAL PREPARATION OF DIMEBON

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Topical treatment of allergic dermatitis is one of the principle problems in modern dermatology. Dimebon, an antihistamine drug, offers anti-allergic local medicinal potential. Dimebon induces anti-allergic effects via multiple mechanisms: blocking of H_1-histamine receptors and partially blocking muscarinic acetylcholine and serotonin receptors. Until now, Dimebon has not been utilized topically. Aim of this investigation was (a) preparation of Dimebon cream, (b) choice of additive substances, (c) technological optimisation of the dermatological cream. We used biopharmaceutical in vitro and pharmacological tests. The detailed biopharmaceutical research used dialysis though a membrane. Polyethylene glycol 400, 4000, carbopol 940, collagen, methyl cellulose and the complex of chitosan-flocare were used as base-carriers. Flocare was used as an emulsifying, solubilizing additive substance. The degree of release of the active component (Dimebon) was determined by measuring optimal density of the solution after regular periods of dialysis. Investigations of this kind were carried out using the choice of the optimal penetrator, and the following additive compounds were used: propylene glycol, glycyrram, polyethylene glycol 400. Investigations to select the appropriate conserving agent were carried out using standard culture of microorganisms, such as: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*. The investigation resulted with the complex of chitosan-flocare being selected as an optimal base, polyethylene glycol 400 as a penetrator, and benzalconium chloride as the conserving agent. The pharmacological methods of investigation had distinct positive results. Thus, the first preparation of an anti-allergic cream with Dimebon has been made. This cream can be used in allergic skin conditions.

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POSSIBILITIES OF USING DIMEBON-GEL WITH ANTIHISTAMINIC ACTIVITY IN DERMATOLOGY

A.V. Majorova, K.N. Koryanova, E.F. Stepanova, I.A. Savenko

Currently cosmetic production is an important consideration of the pharmaceutical market. In Russia there is an increase in the prevalence of dermatological cellular tissue disease. The tendency of this increase is stimulating the investigations into new medicinal products for dermatological conditions. The aim of this investigation was to examine the use of a dermatological medicine, namely the antihistamine compound Dimebon. Dimebon is a first generation histamine H_1 antagonist which was synthesized in Russia. It is a derivate of carboline, which is used as a therapy for various dermatological conditions such as oedema, itching, eczema and some reactions resulting from insect-bites. Here we used chitosan-aminosaccharide which is able to heal the injured skin. In cosmetics, highly-astringent hydro-gels of chitosan are used because they exhibit high penetration into the skin, as well as having immune-modulating, anti-microbial and antifungal properties. Another frequently used compound in dermatological medicines, perfume-production and cosmetics as a hydro-filling base is polyethylene glycol 400 and it is relatively non-toxic. Benzalconium chloride has antiseptic and preserving properties. We have used in vitro biopharmaceutical methods for our investigations. Additionally we have carried out pharmacological tests to study the anti-allergic action of the Dimebon-gel. As a result of our comparative investigations, we have found that the best ones use chitosan. The Dimebon-gel can be used as an antiallergic treatment in complex therapy and treatment of dermatological conditions resulting from the use of some cosmetic products**.** Therefore the use of the Dimebon-gel appears to be very promising in dermatological therapy.

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Chemical aspects of histamine, its receptors and enzymes

NEW TOOLS FOR STUDYING OLD QUESTIONS: ANTIBODIES FOR HISTAMINE METABOLIZING ENZYMES

H.G. Schwelberger, J. Feurle, G. Houen

Many findings on histamine metabolism obtained in animal models have yet to be confirmed in humans, which is mostly due to lack of suitable tools for studying histamine formation and inactivation in man. Therefore, we aimed to produce highly sensitive and specific monoclonal antibodies for human histamine metabolizing enzymes that facilitate the detection and quantitation of the respective proteins at the cellular and subcellular level.

Complete and partial cDNAs encoding human diamine oxidase (DAO) and histamine *N*-methyltransferase (HMT) were expressed as GST fusions in *E. coli*, purified to homogeneity and used for immunization of mice to obtain monoclonal antibodies. These antibodies were screened for specificity and sensitivity and then used to analyze the expression and localization of DAO and HMT in human tissue sections and homogenates by immunohistochemistry and immunoblotting.

Five different monoclonal antibodies specific for human DAO were obtained that can detect DAO with 100-fold greater sensitivity than the most sensitive enzymatic assays currently available. Using these antibodies allowed us to confirm the expression and cellular localization of DAO in various human tissues such as kidney, intestine and placenta where the presence of the enzyme had previously been deduced from activity measurement and DAO mRNA analysis. Due to the high sensitivity, DAO was also detected in biological fluids that previously evaded unequivocal proof of DAO enzymatic activity such as urine. Monoclonal antibodies specific for human HMT are currently being tested and results on the expression of HMT in human tissues should be available shortly.

New monoclonal antibodies not only allow a comprehensive quantitative evaluation of the expression of histamine metabolizing enzymes at the cellular level in man but will also facilitate sensitive analyses of disease associated alterations of these proteins.

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CHARACTERIZATION OF DIAMINE OXIDASE FROM HUMAN SEMINAL PLASMA

H.G. Schwelberger, J. Feurle

The preparation and characterization of native human diamine oxidase (DAO) is difficult because the enzyme is expressed at relatively low levels in man, and healthy human tissues containing DAO are difficult to obtain in sufficient quantity. Reports in the older literature indicating a considerable DAO activity in human seminal plasma prompted us to purify and characterize the enzyme from this readily available source.

Human seminal plasma, the cell-free fraction of the semen, was fractionated by consecutive affinity binding on Heparin Sepharose, hydrophobic interaction on Phenyl Sepharose, anion exchange on CIM-QA, and size fractionation on Superdex 200 to obtain a nearly homogenous DAO protein. DAO was characterized enzymatically and by various gel electrophoretic techniques.

DAO was purified from human seminal plasma ca. 300-fold with ca. 20% yield. The purified protein appears homogenous on two-dimensional IEF/PAG and monomeric seminal plasma DAO migrates at ca. 100 kDa similar to kidney DAO but is slightly smaller than placenta DAO, which is due to different glycosylation of the protein in different tissues. The enzymatic properties of seminal plasma DAO are very similar to DAO proteins from other human tissues with histamine and aliphatic diamines being the preferred substrates and significant conversion of polyamines at higher substrate concentrations.

Human seminal plasma DAO can rapidly be purified and is enzymatically and electrophoretically similar to DAO proteins from other human tissues. Likely sources of seminal plasma DAO are the prostate and testis where DAO expression is detectable at the mRNA level. The functional importance of DAO in the semen remains to be determined.

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BIOISOSTERIC REPLACEMENT OF IMIDAZOLE IN CYANOGUANIDINE-TYPE hH_4R AGONISTS—AN APPROACH TO IMPROVE H_4R SELECTIVITY

R. Geyer, P. Igel, A. Buschauer

The human histamine H_4 receptor (hH_4R) is considered a potential drug target, for instance, for treatment of inflammatory and allergic diseases. Potent and selective ligands including agonists are required as pharmacological tools. Many agonists show only low or insufficient H_4R selectivity. Also the investigation in animal models is hampered by species-dependent discrepancies regarding potencies and histamine receptor subtype selectivities.

Very recently, we identified imidazolylalkylcyanoguanidines as highly potent and selective hH_4R

agonists. Highest potency resided in compounds with a tetramethylene linker, connecting the imidazole and the cyanoguanidine moiety as in UR-PI376. Introducing a conformationally restricted cyclopentylene linker further increased H_4R agonistic potency and selectivity as in *trans-(+)*-UR-RG98. Nevertheless, these compounds have still some affinity for histamine receptors other than the H_4R. Therefore, by analogy with a strategy which was very successful in the H_2R agonist field, we applied a bioisosteric approach, aiming at H_4R agonists with further improved selectivity, and synthesized a series of hetarylbutylcyanoguanidines containing, for instance, aminotriazoles or aminopyrimidines as potential bioisosteres of the imidazole ring.

Potencies, intrinsic activities and selectivities of these compounds were determined in GTPγS assays using membrane preparations of *Sf9* insect cells, expressing the respective hHR subtype ($H_{3/4}R$). Unfortunately, only a few of the investigated compounds showed relevant H_4R activity. Even minor variations like the introduction of a methyl group in 2- or 5-position of the imidazole ring, resulted in a dramatic decrease in H_4R agonistic activity. Although the bioisosteric replacement of imidazole did not yield potent H_4R ligands so far, the synthesized compound library will be useful in molecular modelling studies to refine the H_4R receptor model and to design new H_4R agonists.

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SECOND AND THIRD EXTRACELLULAR LOOPS OF THE HISTAMINE H_4 RECEPTOR ARE INVOLVED IN RECEPTOR ACTIVATION

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A recent study from our laboratory revealed substantial differences in pharmacological profile of human (h) and canine (c) histamine H_4 receptor (H_4R). Surprisingly, thioperamide and 1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120), acting as inverse agonists at hH_4R via $G_{i/o}$ coupling, exhibited partial agonistic activity at cH_4R.

In order to elucidate the contribution of extracellular regions to species differences between hH_4R and cH_4R, we generated chimeric receptors by replacing corresponding domains of hH_4R with canine N-terminus ($h_{cNT}H_4R$) and three canine extracellular loops, respectively ($h_{cE1}H_4R$, $h_{cE2}H_4R$ and $h_{cE3}H_4R$). Receptors were expressed in Sf9 insect cells and subsequently characterised in [^{3}H] histamine binding experiments and in steady-state GTPase activity assays, where histamine, 5-methylhistamine, thioperamide, JNJ7777120 and clozapine were examined.

The exchange of N-terminus or first extracellular loop did not affect hH_4R pharmacology. The influence of altered second or third extracellular loop of hH_4R on potency of examined ligands was ligand-specific rather than agonist-/inverse agonist-specific. Moreover, almost full inverse agonistic activity of thioperamide at hH_4R was strongly reduced at $h_{cE2}H_4R$ and $h_{cE3}H_4R$. Most strikingly, JNJ7777120, a weak inverse agonist at hH_4R, exhibited partial agonistic activity at $h_{cE2}H_4R$ and $h_{cE3}H_4R$. Molecular dynamic simulations suggest that the second and the third extracellular loop are, independently of each other, involved in partial/inverse agonism of JNJ7777120. Furthermore, the second as well as third extracellular loops do not directly interact with JNJ7777120 in the binding pocket. In conclusion, the second and third extracellular loops of the H_4R are involved in the receptor activation process.

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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF BIVALENT ACYLGUANIDINE-TYPE HISTAMINE H_2 RECEPTOR AGONISTS

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N^G-Acylated hetarylpropylguanidines represent a new class of potent histamine H_2 receptor (H_2R) agonists. Very recently, a tremendous increase in potency was achieved by applying the bivalent ligand approach to acylguanidine-type H_2R agonists. The combination of two hetarylpropylguanidines by alkanedioyl spacers with 6–8 methylene groups led to the most potent H_2R agonists known so far: These compounds proved to be up to two orders of magnitude superior to the monovalent acylguanidines and up to 4,000-fold more potent than histamine.

The present study was focused on the chemical nature of the spacer as well as on "unsymmetrical bivalent ligands" bearing two different sets of pharmacophore groups. Therefore, various bivalent acylguanidines were synthesized by connecting the guanidine groups of two molecules with different diacyl spacer. The synthesized compounds were pharmacologically investigated for H_2R agonism at isolated guinea pig (gp) right atria and in steady-state GTPase assays using human (h) and gpH_2R-$G_{s\alpha S}$ fusion proteins. Moreover, the histamine receptor selectivity

profile (H_2R vs. H_1R, H_3R, H_4R) was determined (GTPase assays).

This study demonstrates the structural requirements of bivalent acylguanidine-type H_2R agonists and substantiates previous results obtained with symmetrical compounds, suggesting that the gain in potency is due to the interaction with an additional binding site at the same receptor molecule rather than by bridging the binding pockets of a hypothetical receptor dimer. The synthesized compounds are promising pharmacological tools for further investigations of hypothetical H_2R dimers. In this respect, successful approaches and results from the H_2R field may be exemplary for and applicable to other GPCRs.

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(UN-)SUBSTITUTED ARYLTRIAZINE DERIVATIVES AS HISTAMINE H_4 RECEPTOR LIGANDS

T. Karcz, J. Handzlik, J. Ner, T. Kottke, S. Schwed, R. Seifert, H. Stark, K. Kieć-Kononowicz

The histamine H_4 receptor (H_4R) appears to be a prominent target for drug development. It is mainly expressed in bone marrow as well as in peripheral leukocytes and is claimed to play an important role in a wide variety of immunological and inflammatory processes. Therefore, the potential H_4R ligands could provide innovative therapies for different immuno-based diseases.

In our recent studies, we considered the 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine structure as a scaffold for development of novel histamine H_4 receptor ligands, based on promising pharmacological results for structurally related compounds.

The present work is the continuation of structure–activity relationships investigation in the group of triazine derivatives. Fourteen derivatives with a diversity of substituents placed in different positions of aryl ring were obtained. Within the discussed study, affinity data were collected in radioligand binding assays, using the model of S*f*9 insect cells, transiently expressing recombinant human histamine H_4 receptors. In addition the drug-likeness of tested compounds has been estimated by means of computational analysis.

The evaluated series of compounds showed in vitro affinities in the micromolar and submicromolar concentration range. The affinity of the compounds showed great susceptibility to the kind and pattern of aryl ring substitution. The K_i value obtained for the most potent compound was 203 ± 65 nM.

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CHLOROPHENOXYL DERIVATIVES AS HISTAMINE H_3R ANTAGONISTS

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The H_3R is involved in the central and peripheral regulation of levels of histamine and other neurotransmitters (e.g. acetylcholine, noradrenaline, dopamine, serotonin, GABA, glutamate and substance P). Intensive pharmacological studies suggest the utility of H_3R antagonists/inverse agonists in CNS diseases (e.g. narcolepsy, ADHD, Alzheimer's disease, schizophrenia, epilepsy), allergic rhinitis, obesity and pain. Many pharmaceutical companies and academic researchers have synthesized a large number of highly potent H_3R antagonists/inverse agonists. Some of the compounds are undergoing evaluation in clinical development. Especially interesting is pitolisant (1-{3-[-3{-4-chlorophenyl) propoxy]propyl}piperidine), which is progressing through phase III clinical trials.

The present work is a continuation of our previous works in the histamine H_3R field. As the lead structure for these studies, we have chosen pitolisant and 3- or 4-chlorophenoxy analogues of this compound have been prepared. Compounds with four secondary amines as a basic group (piperidine, 3- or 4-methylpiperidine and azepine) have been synthesized. A length of the alkyl spacer between an amine moiety and a chlorophenoxy ring has been also changed. The desired compounds were obtained by the direct reaction of prepared chlorophenoxyalkyl bromides with corresponding amines.

Histamine H_3R affinities of the synthesized compounds were evaluated at the human receptor. Displacement of [^{3}H] N^{α}-methylhistamine from HEK-293 cells stably expressing hH$_3$R was measured. Most compounds showed moderate histamine H_3R affinities (hH$_3$R 100 nM < hKi < 500 nM).

Generally, the most potent were 3-methylpiperidine derivatives and the length of the alkyl chain influenced the H_3R potency.

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

CONTRIBUTION OF THE VASCULAR ENDOTHELIUM IN THE HISTAMINE H_3 AND H_4 RECEPTOR-MEDIATED EFFECTS IN BLOOD VESSELS IN ADJUVANT ARTHRITIS

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This study sought to investigate the involvement of the vascular endothelium in the effects mediated by H_3 or H_4 receptors (H_xR) in large arteries and veins in rats with adjuvant arthritis. Male Wistar rats of ~300 g b.w. received complete Freund's adjuvant (CFA) i.d. and/or 1 and 3 mg/kg or 10 and 30 mg/kg i.p. of the H_3R and H_4R inverse agonists/antagonists GSK334429 (AD Medhurst, GSK, Essex, UK) and JNJ7777120 (RL Thurmond, JNJ, CA, USA), respectively. Following sacrifice at day 20, abdominal aorta (AA) and inferior vena cava (IVC) were dissected out. Endothelium was removed by 30 s perfusion with sodium deoxycholate, using a short Abbocath 22 intravenous catheter. Tissue histamine either in the presence or following removal of the endothelium was quantified fluorophotometrically. CFA administration resulted in the development of arthritic signs in animal paws. Statistically significant reductions in histamine levels in both AA and IVC were observed following JNJ7777120 administration, while GSK334429 tended to decrease histamine levels in the IVC of CFA-treated animals. Upon removal of the vascular endothelium histamine was not detectable in the tissues. The results provide indication for the implication of the vascular endothelium in the H_3R/H_4R-mediated automodulatory action of histamine in large blood vessels. The role of H_3R and/or H_4R in shaping the (patho)physiological vascular tone and systemic inflammatory responses in blood vessels is currently under investigation.

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SARCO(ENDO)PLASMIC RETICULUM CA(2 +)ATPASE (SERCA) EXPRESSION IN EXPERIMENTAL HEART ISCHEMIA–REPERFUSION INJURY: EFFECTS OF HISTAMINE AND HISTAMINE RECEPTOR LIGANDS

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Histamine (HA) exerts inotropic, chronotropic and arrhythmogenic effects. Sequestration of calcium by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) mediates muscle relaxation.

SERCA expression was examined immunohistochemically in guinea pig isolated hearts subjected to ischemia–reperfusion under conditions corresponding to those in cardiopulmonary bypass surgery in humans. Hearts were taken from male guinea pigs (350–450 g) and perfused by the Langendorff technique in the sequence: initial perfusion (non-working and working heart, each 15 min), 3 min perfusion with cardioplegic fluid to stop the heart, 40 min ischemia, and reperfusion (non-working and working heart, each 15 min). Hemodynamic and metabolic parameters were recorded by standard techniques. SERCA was visualised in left ventricle specimens by SERCA2 monoclonal antibodies (NCL-SERCA2, Clone IID8, 1:400 Novocastra) with a LSAB + HRP (DAKO) detection kit and DAB chromogen.

HA and all tested ligands altered cardiac rhythm, the changes being transient and disappearing with reperfusion, except in the case of mepyramine where the alterations became more intense and led to ventricular arrest. HA and H_2 agonists increased oxygen consumption (>2×), efflux of HCO_3^- (>3×) and H^+ (>4×). HA dose-dependently increased SERCA activity as visualised by immunostaining. Ciproxifan acted similarly, but weakly, while 2-(3-trifluoromethylphenyl)histamine, mepyramine, cimetidine and R-α-methylhistamine led to reduced enzyme expression. No changes were found with 2-(2-pyridyl)-ethylamine or dimaprit. The SERCA expression scores correlated with coronary flow, cardiac output, heart rate, oxygen consumption, H^+ and HCO_3^- efflux.

Thus, HA may increase postischemic heart efficiency by increasing SERCA activity. The effect may be mediated via H_2 and H_3 receptors, acting in opposition. SERCA activation by HA rather than any of H_1–H_3 receptors may account for increased aerobic and anaerobic heart metabolism during hypoxia.

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IMOPROXIFAN MODULARES AND IL-6 AND IL-10 PRODUCTION BY PBMC IN PATIENTS WITH Q-POSITIVE MYOCARDIAL INFARCTION

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Inflammatory mechanisms have an important role in the pathogenesis of ischemic heart disease (IHD) and the occurrence of acute ischemic syndromes in some cases results in acute Q-positive myocardial infarction (Q-AMI).

Recently, research has focused on the roles of histamine and cytokine activation in the development IHD and AMI. The purpose of this study was to investigate the impact of H_3/H_4 receptors in the modulation of pro-and anti-inflammatory cytokine (IL-6 and IL-10) production by PBMC of patients with Q-AMI. PBMC of 20 Q-AMI patients and 20 healthy volunteers were cultivated with and without the H_3/H_4 antagonist, Imoproxifan. The concentrations of IL-10 and IL-6 were measured be ELISA. It was shown that the spontaneous production of IL-6 was greater ($p = 0.03$) in patients—12.3 (5.3; 17.4) than in healthy donors—4.2 (1.4; 6.3); data are given as median (25; 75 percentiles). The spontaneous production of IL-10, on the contrary was lowered—2.1 (1; 3.9) in comparison with control—9.8 (5.2; 12.7) pg/ml. Imoproxifan at a high concentration (10^{-5} M) significantly reduced ($p = 0.02$) IL-6 production from 12.3 to 5.1 (1.3; 7.6) pg/ml and increased IL-10 synthesis up to 14.3 (10.3; 20.6) pg/ml ($p = 0.04$). Thus, it was established that histamine via H_3/H_4 receptors may be involved in cytokine production in patients with Q-AMI and may have a possible role in the modulation of the pro- and anti-inflammatory cytokine balance.

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THE INFLUENCE OF EXOGENOUS SALSOLINOL ON MAST CELLS, INTERSTITIAL CELLS OF CAJAL AND MYENTERIC PLEXUS NEURONS IN THE RAT GUT

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Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is a representative of catechol isoquinoline derivatives used to induce parkinsonism in rodents. Gastrointestinal (GI) impairment is one of the most critical non-motor features of Parkinson's disease (PD).

Mast cells (MC) in the GI tract have been found in close spatial contact with interstitial cells of Cajal (ICC), recognized as pacemaker cells, and myenteric plexus neurons (MPN), suggesting their functional interaction. Damage to, or change in activity of, these cells may result in severe dysfunction in gut motility. The aim of this study was to evaluate the effects of salsolinol on MC, intramuscular ICC and MPN in the rat gastrointestinal tract.

Male Wistar rats ($n = 10$) were treated with salsolinol (50 mg/kg/day i.p.) for 3 weeks and an equivalent group served as a control. Fragments of stomach, duodenum and large intestine were removed and paraffin-embedded specimens and longitudinal muscle–myenteric plexus strips were prepared. The total numbers of MC were evaluated by toluidene blue staining. Gastric antral, duodenal and ascending colon intramuscular ICC (anti-c-Kit antibody) and MPN (anti-PGP9.5 antibody) were assessed by image analysis.

The total number of MC in the GI wall of the stomach (98.7 ± 53.3 vs. 156.7 ± 45.8), duodenum (2.6 ± 2.1 vs. 7.8 ± 7.8) and colon (12.8 ± 14.0 vs. 10.7 ± 17.1) were decreased in the salsolinol group compared to the control. The area of PGP 9.5-positive cells was lower in all examined strips of GI wall in comparison with the control group (by 51, 55, 45%, respectively). The area of cells stained with anti-c-kit (i.e. cells of Cajal) was lower in the stomach and duodenum (by 28, 19%, respectively) but no differences were observed in the colon.

Salsolinol thus exerts its destructive influence over MC, MPN and ICC in all examined segments of the GI tract. This may lead to a GI dysfunction. These results may then provide new insight into the pathophysiology of PD.

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HISTAMINE MODULATES BxPC3 CELL LINE GROWTH AND THE INTERACTION WITH MICROENVIRONMENT

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We have demonstrated that histamine (HA) regulates processes related to cell proliferation, differentiation and invasive ability via the four membrane receptors in various tumor cell lines. The aim here was to assess the role of HA in a human pancreatic adenocarcinoma cell line BxPC3.

We determined that HA modulates cell proliferation using a clonogenic assay. A rise was observed at low doses of HA (0.5–1 μM) while a decrease was found >20 μM (182% $p < 0.01$ and 65% $p < 0.05$). A stimulatory effect was evoked by the H_3 agonist (R)-α-methylhistamine (151% $p < 0.05$) and the H_2 agonist amthamine (160% $p < 0.05$); both responses were blocked by the specific antagonists JNJ5207852 and ranitidine, respectively. Bromodeoxiuridin (BrdU) incorporation evaluated by immunostaining (HA vs. C, $p < 0.05$) and cyclin D and E expression by immunoblot confirmed the increase in number of cells in S phase of cell cycle when cultures received low doses of HA or amthamine or (R)-α-methylhistamine. Conversely the H_1 agonist 2-(3-trifluormethylphenyl) histamine and H_4 agonists VUF 8430 and clobenpropit inhibited cell proliferation (60% $p < 0.05$) by increasing apoptosis.

Matrix metalloproteinases secretion and cell migration are critical for tumor invasion. We found that HA dose

dependently enhances MMP9 activity assessed by zymography (up to 225% $p < 0.01$) and cell migration by transwell systems (up to 200% $p < 0.05$).

BxPC3 cells (s.c.) in nude mice led to the development of differentiated tumors. Tumors xenografted in HA treated animals showed a higher growth rate (tumor doubling time: 9 vs. 15 days, $p < 0.05$) and a more important stromal desmoplastic reaction than control animals. In vitro studies determined that conditioned media from HA treated BxPC3 cells, increased proliferation of a normal fibroblast cell line, assayed by PCNA expression and BrdU incorporation (210% $p < 0.01$).

Pancreatic cancer has a high mortality rate. Thus drugs that can both regulate tumor cell survival and metastatic ability will help to delineate more effective strategies for therapeutic intervention.

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HISTAMINE H_4 RECEPTOR EXPRESSON IN HUMAN NEVUS AND MELANOMA

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Malignant melanoma is an aggressive, therapy-resistant malignancy of melanocytes. The incidence of melanoma has been steadily growing worldwide, resulting in an increasing public health problem. The expression of all histamine receptors (HR) subtypes were demonstrated in human melanoma cell lines. We have previously shown that histamine inhibits proliferation and migration, and induces differentiation and senescence through the activation of the H_4R in WM35 primary and M1/15 highly metastatic human melanoma cells. The aim of this work was to investigate the presence of H_4R in human nevus and melanoma skin biopsies and its association with proliferating cell nuclear antigen (PCNA), histamine and histidine decarboxylase (HDC) expression levels by immunohistochemistry. The immunohistochemical analysis showed that H_4R was detected in 42% (8/19) of melanoma samples and in 83% (15/18) of nevi, in which a higher level of expression was observed ($^*p = 0.0107$, Mann–Whitney's two tail test). We additionally evaluated PCNA immunostaining as an indicator of active proliferation, and results demonstrated that only melanomas expressed PCNA, which inversely correlated with H_4R expression ($^*p = 0.0158$; Sperman $r = -0.54$). Melanoma tissues demonstrated higher levels of HDC expression compared to nevi, while no difference was observed in histamine content. Present findings indicate that the H_4R is expressed in human melanoma and nevus biopsies, confirming that the H_4R is present not only in cell lines but also in human skin tissue. The identification of H_4R and the clarification of its role in human malignant melanoma progression may contribute to the advancement in the treatment of this disease.

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HISTAMINE RECEPTOR LIGANDS AS POTENTIAL RADIOSENSITIZERS OF HUMAN BREAST CANCER CELLS

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We have previously demonstrated that histamine (HA) significantly protects healthy tissues against ionizing radiation damage in rodents. The aim here was to investigate whether HA and its ligands could modulate in vitro radiosensitivity of two breast cancer cell lines, MDA-MB-231 [estrogen receptor (ER)$\alpha-$] and MCF-7 (ER$\alpha+$). Thus we evaluated the response to gamma radiation using a Cesium 137 source (0–10 Gy). Cells were treated with HA and/or specific ligands 24 h before irradiation and radiobiological parameters, including 2 Gy surviving fraction (SF2 Gy), were obtained from the survival curves adjusted to linear quadratic model [$SF = e^{-(\alpha D+\beta D2)}$]. Cell apoptosis was determined by the TUNEL assay and Annexin-V staining, while senescence was investigated through the activity of β-galactosidase after 2 Gy dose. Catalase activity and peroxide levels were measured spectrophotometrically and by flow cytometry, respectively. Results indicate that HA increased radiosensitivity of MDA-MB-231 (SF2 Gy 0.06 ± 0.02 vs. 0.22 ± 0.04, $p < 0.01$) and MCF-7 cells (FS2 Gy 0.16 ± 0.01 vs. 0.21 ± 0.02, $p < 0.05$). In MDA-MB-231 cells, this effect was mimicked by the H_1R agonist 2-(3-(trifluoromethyl)phenyl)histamine (SF2 Gy 0.04 ± 0.01) while was blocked by the combined treatment with mepyramine. In MCF-7 cells, the main receptor involved was the H_4R, since the H_4R agonists (clobenpropit and VUF8430) reduced the SF 2 GY (0.06 ± 0.02) and JNJ7777120 treatment reversed HA effect. Radiation increased cell apoptosis and senescence, responses that were enhanced by HA and its agonists. Also in MDA-MB-231 cells, HA modulated peroxide levels and catalase activity via the H_1R. We conclude that HA modulates radiosensitivity of

breast cancer cell lines through different receptor subtypes, suggesting that the combined use with radiation could be an attractive strategy to enhance the efficacy of radiotherapy for breast cancer treatment.

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