INTERNATIONAL SCIENTIFIC CONFERENCE
OF THE BULGARIAN PEPTIDE SOCIETY

27-29 August 2021, Velingrad, Bulgaria

ABSTRACT BOOK

Supported by Grant КП-06-МНФ/42/16.12.2019 from the National Science Fund in Bulgaria; European Peptide Society; Bulgarian Peptide Society; Institute of Neurobiology at the Bulgarian Academy of Sciences; University of Chemical Technology and Metallurgy; ELTA 90

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Friday, 27th August 2021

16.00 – 18.15	**Registration**
18.15 – 18.30	**WELCOME WORDS:** ***in Kleptuza Hall*** Chairperson of the Organizing Committee Prof. Reni Kalfin President of the Bulgarian Peptide Society Prof. Emilia Naydenova
SESSION 1.	**PLENARY LECTURES** ***in Kleptuza Hall*** **CHAIRPERSONS: EMILIA NAYDENOVA AND RENI KALFIN**
18.30 – 19.00	**Anna Maria Papini (Italy)** "Aberrantly modified conformational peptide epitopes specific for antibodies, biomarkers of immune-mediated diseases, can be reminiscent of an early infection: the chicken or egg dilemma?"
19.00 – 19.30	**Illana Gozes (Israel)** "Peptide-based drug development based on the autism/Alzheimer's-linked activity-dependent neuroprotective protein (ADNP)"
19.30 – 20.00	**Paula Gomesh (Portugal)** "Antimicrobial peptide-based strategies to tackle chronic skin and soft tissue infections"
20.30 – 22.30	**WELCOME COCKTAIL**

Saturday, 28th August 2021

SESSION 2.	**DRUG DESIGN AND BIOLOGICAL ACTIVITY OF PEPTIDES: ORAL PRESENTATIONS** ***in Jundola Hall*** **CHAIRPERSON: TAMARA PAJPANOVA**
9.30 – 9.50	**Tamara Pajpanova (Bulgaria)** "Unnatural amino acids as a privileged class of building blocks in drug design"
9.50 – 10.10	**Vasile Robert Gradinaru, Cosmin Stefan Mocanu, Brindusa Alina Petre, Laura Darie Ion, Gabi Drochioiu, Mihaela Homocianu, Loredana Elena Nita (Romania)** "A new bioinspired peptide with potential self-assembly properties"
10.10 – 10.30	**Andreas Tzakos (Greece) "**Design and development of novel theranostic agents exploiting the malignant tumor microenvironment"

10.30 – 10.50	**Selma Arzu Vardar (Turkey) "**Exercise induced alterations of circulatory levels of heat shock proteins"
10.50 – 11.10	**Nurettin Aydogdu (Turkey)** "Irisin and its physiological role in the kidney"
11.10 – 11.40	**COFFEE BREAK**
11.40 – 12.00	**Lubomir Vesenkov (France)** "Organized oligomers as vectors for cell penetration"
12.00 - 12.20	**Tatyana Dzimbova, Fatima Sapundzhi, Nevena Pencheva, Peter Milanov (Bulgaria)** "Analysis of the docking results of some selective MOR and DOR ligands"
12.20 – 12.40	**Petya Hadzhibozheva, L. Pashova-Stoyanova, Zh. Tsokeva, M. Ganeva, K. Nancheva, G. Ilieva, V. Nanchev, A. Tolekova, Ts. Georgiev (Bulgaria)** "Appetite-related peptides in fructose-drinking rat models"

13.00 – 14.00 LUNCH BREAK

SESSION 3. BIOLOGICAL ACTIVITY OF PEPTIDES: ORAL PRESENTATIONS *in Jundola Hall* **CHAIRPERSON: PAVLINA DOLASHKA**	
14.00 – 14.20	**Alessandro Pesaresi (Italy), Doriano Lamba, Lyubomir T. Vezenkov, Daniela S. Tsekova** "Structure-actitivty relationship of multitarget directed galanthamine-peptide derivatives designed for the treatment of Alzheimer's desease"
14.20 – 14.40	**Lyudmila Velkova, Aleksandar Dolashki, Ventseslav Atanasov, Dimitar Kaynarov, Asya Daskalova, Pavlina Dolashka (Bulgaria)** "Study of novel antimicrobial peptides and glycopeptides in *Cornu aspersum* mucus"
14.40 – 15.00	**Sirine Jaber (Lebanon), Emilia Naydenova, Tsvetelina Angelova, Veronica Nemska, Ivan Iliev, Inna Solikovska, Nelly Georgieva, Dancho Danalev (Bulgaria)** "Synthesis, anticancer and antimicrobial activity of shortened analogues of $(KLAKLAK)_2$"
15.00 – 15.20	**Mihaela Belouhova, Yana Topalova, Elmira Daskalova, Lyudmila Velkova, Pavlina Dolashka (Bulgaria)** "Antibacterial effect of two peptides/proteins fractions from snail mucus – fluorescent and digital image analysis"
15.30 – 16.00	**COFFEE BREAK**
16.00 – 16.20	**Asya Daskalova, Lyudmila Velkova, Aleksandar Dolashki, Dimitar Kaynarov, Ventseslav Atanasov, Pavlina Dolashka (Bulgaria)** "Investigation of conformational changes in bioactive fractions and peptides from mucus of *Cornu aspersum* by fluorescence spectroscopy"
16.20 – 16.40	**Momchil Kermedchiev, Konstantin Angelov, Alexandar Dolashki, Lyudmila Velkova, Pavlina Dolashka (Bulgaria)** "Treatment of wounds with natural substances"
16.40 – 17.00	**PRESENTATION ELTA Company**

SESSION 4. PEPTIDE CHEMISTRY: E-POSTER PRESENTATIONS *in Jundola Hall* **CHAIRPERSON: DANCHO DANALEV**	
17.30 – 17.35	**Aleksandar Dolashki, Lyudmila Velkova, Pavlina Dolashka (Bulgaria)** "Novel peptides in the hemolymph of *Cornu aspersum* snail identified by mass spectrometry"
17.35 – 17.40	**Nikolay G. Vassilev, Svetlana Simova, Miroslav Dangalov, Lyudmila Velkova, Venceslav Atanassov, Aleksandar Dolashki, Pavlina Dolashka (Bulgaria)** "Comparatively study of metabolites profiling of *Helix aspersa* mucus, *Helix lucorum* hemolymph and *Rapana venosa* hemolymph using ^{1}H NMR and mass spectrometry"
17.40 – 17.45	**Dimitar Kaynarov, Lyudmila Velkova, Aleksandar Dolashki, Asya Daskalova, Ventseslav Atanasov, Pavlina Dolashka (Bulgaria)** "Identification of novel glycopeptides identified in the mucus of garden snail *Cornu aspersum* by mass spectrometry"
17.45 – 17.50	**Aleksandar Dolashki, Lyudmila Velkova, Dimitar Kaynarov, Ventseslav Atanassov, Asya Daskalova, Pavlina Dolashka (Bulgaria)** "Determination of the conformational stability of peptides from *Helix aspersa* snail by circular dichroism"
17.50 – 17.55	**Yuliana Raynova, D. Yancheva, S. Todinova, M. Guncheva, K. Idakieva (Bulgaria)** "Conformation analysis of modified *Helix lucorum* hemocyanin"
17.55 – 18.00	**Veronika Karadjova, Dessislava Borisova, Spaska Yaneva, Emilia Naydenova, Trayana Dolchinkova, Vladislava Ivanova, Dancho Danalev (Bulgaria)** "Synthesis and characterization of copper complexes with peptide ligand as potential anticancer agents"
18.00 – 18.05	**Aleksandra Tencheva, Radoslav Chayrov, Ivanka Stankova (Bulgaria)** "Amantadine and rimantadine analogues with glycine based amino acids and small peptides"

20.00 – 23.00 GALA DINNER

Sunday, 29th August 2021

SESSION 5. BIOLOGICAL ACTIVITY OF PEPTIDES: E-POSTER PRESENTATIONS *in Jundola Hall* **CHAIRPERSON: IVANKA STOINEVA**	
9.30 – 9.35	**Tanya Dimova, P. Rashev, S. Manchev, D. Tsekova , L. Vezenkov (Bulgaria)** "*In vivo* and *in vitro* evaluation of the anti-inflammatory potential of 4-aminopyridine derivatives"
9.35 – 9.40	**Boryana Yakimova, L. Dobreva, P. Kardaleva, S. Danova, I. Stoineva (Bulgaria)** "New natural and synthetic peptides as positive impact on hypertension"
9.40 – 9.45	**Lubomir Petrov, Mihail Kachaunov, Albena Alexandrova, Elina Tsvetanova, Almira Georgieva, Lyudmila Velkova, Ventssislav Atanasov, Aleksander Dolashki, Pavlina Dolashka (Bulgaria)** "Antioxidant activity of hemolymph isolated from *Rapana venosa*"

9.45 – 9.50	**Maria Lazarova, Lyubka Tancheva, Lyudmila Velkova, Alexander Dolashki, Diamara Uzunova, Petya Gavrilova, Krasimira Tasheva, Teodora Taseva, Yordan Hodzhev, Atanas G. Atanasov, Miroslava Stefanova, Ventseslav Atanasov, Reni Kalfin, Pavlina Dolashka (Bulgaria)** "Snail (*Helix aspersa*) extract improves impaired spatial learning and memory in Alzheimer's type of dementia by regulating CREB/BDNF signaling"
9.50 – 9.55	**Elina Tsvetanova, Albena Alexandrova, Almira Georgieva, Lyubka Tancheva, Maria Lazarova, Reni Kalfin, Miroslava Stefanova, Alexander Dolashki, Lyudmila Velkova, Pavlina Dolashka (Bulgaria)** "Neuroprotective and antioxidant effect of fresh snail extract (*Helix aspersa*) in rat's brain in Alzheimer's type of dementia"
9.55 – 10.00	**Maya Chochkova, Anna-Maria Hristova, Boyka Stoykova, Yuhuan Li, Iva Tsvetkova, Hristo Najdenski, Yavor Mitrev, Martin Štícha (Bulgaria)** "In vitro antimicrobial investigations of substituted cinnamic acid amides"
10.00 – 10.05	**Aneliya Balacheva, Roumyana Detcheva, Tamara Pajpanova (Bulgaria)** "Antiproliferative effect of novel RGD peptide analogues"
10.05 – 10.10	**Proletina Kardaleva, Vadimir Vanik, Zuzana Gazova, Diana Fedunova, Denitsa Panteleeva, Maya Guncheva (Bulgaria)** "Effect of amino acid-based ionic liquids on insulin amyloid aggregation"
10.10 – 10.15	**Ekaterina Krumova, Pavlina Dolashka, Nedelina Kostadinova, Radoslav Abrashev, Jeny Miteva-Staleva, Aleksander Dolashki, Lyudmila Velkova, Vladislava Dishlijska, Boryana Spasova, Maria Angelova (Bulgaria)** "Antioxidant properties of a low molecular peptide fraction from the garden snail *Helix aspersa* possessing antifungal activity"
10.15 – 10.20	**Ivanka Stankova, Aleksandra Tencheva, Radoslav Chayrov, Tsvetelina Angelova, Veronica Nemska, Nelly Georgieva, Dancho Danalev (Bulgaria) "**Biological activity of amino acid derivatives of memantine"
10.30 – 11.00	**COFFEE BREAK**
SESSION 6. PEPTIDE CHEMISTRY: E-POSTER PRESENTATIONS *in Jundola Hall* **CHAIRPERSON: NIKOLAY VASSILEV**	
11.00 – 11.05	**Iliyan Ognyanov, Ivan Bogdanov, Maya Georgieva, Martina Peeva, Aneliya Balacheva, Toni Kühl, Tamara Pajpanova, Diana Imhof, Nikolay T. Tzvetkov (Bulgaria)** "Neurotensin (NT8-13) peptide mimetics: virtual multi-target activity estimation and stability studies"
11.05 – 11.10	**Fatima Sapundzhi, Tatyana Dzimbova (Bulgaria)** "Application of molecular docking programs and virtual screening for drug design"
11.10 – 11.15	**Tatyana Dzimbova, Atanas Chapkanov (Bulgaria)** "Combined action of his-leu analogues on angiotensin converting enzyme (ACE) and angiotensin receptor (AR)"
11.15 – 11.20	**Tsonova J, Ivanov I., Naidenova E. (Bulgaria)** "Design and synthesis of new tyrosine kinase inhibitors for the treatment of chronic myeloid leukemia and other malignant diseases"
11.20 – 11.25	**Dessislava Borisova, Emilia Naydenova, Boryana Borisova, Dancho Danalev (Bulgaria)** "Synthesis and characterization of new analogues of octreotide and vapreotide containing citrulline"

11.25 – 11.30	**Petar Todorov, Stela Georgieva, Petia Peneva (Bulgaria)** "Synthesis and characterization of new N-modified hybrid hemorphin analogues"
11.30 – 11.35	**Petar Todorov, Desislava Staneva, Stela Georgieva, Petia Peneva, Ivo Grabchev (Bulgaria)** "Synthesis and characterization of new antimicrobial fluorescent peptides for antivirus protection of textile materials"

SESSION 7. CHEMISTRY AND BIOLOGICAL ACTIVITY: E-POSTER PRESENTATIONS *in Jundola Hall* **CHAIRPERSON: NIKOLAY TZVETKOV**	
12.00 – 12.05	**Cosmin Stefan Mocanu, Vasile Robert Gradinaru, Gabi Drochioiu (Romania)** "Metal ions interaction with amyloid-beta monomer and fibril, a theoretical and experimental approach: Implications for Alzheimer's disease progression"
12.05 – 12.10	**Tzvetelin Georgiev, Petya Hadzhibozheva, Y. Karamalakova, K. Georgieva, F. Perinkadakatt, J. Ananiev (Bulgaria)** "Therapeutic effects of glutathione bioavailability modulation in the light of ferroptosis"
12.10 – 12.15	**Ivelina Himcheva, Emilia Naydenova, Galiya Stavreva, Adriana Bocheva (Bulgaria)** "Participation of the opioidergic and nociceptinergic systems in the effects of nociceptin and new analogues after acute and chronic immobilization stress"
12.15 – 12.20	**Hristina Nocheva, Georgi Bogdanov, Roman Tashev, Rumen Nikolov (Bulgaria)** "The endogenous cannabinoid system and nitric oxide interact in modulation of cold stress-induced analgesia"
12.20 – 12.25	**Roman Tashev, Hristina Nocheva, Margarita Ivanova (Bulgaria)** "Lateralized active avoidance learning and memory to AngII and losartan microinjected into amygdala in rats depression model"
12.25 – 12.30	**Luiza Madalina Gradinaru, Maria Bercea, Robert Vasile Gradinaru (Romania)** "BSA/polymer hybrid hydrogels as carrier for neomycin delivery"

12.30 – 13.00 CLOSING REMARKS AND YOUNG SCIENTIST AWARDS

Acknowledgements: **The International Scientific Conference of the Bulgarian Peptide Society 27-29 August 2021 in Velingrad, Bulgaria was supported by Grant КП-06-МНФ/2019 from the National Science Fund in Bulgaria; European Peptide Society; Bulgarian Peptide Society; Institute of Neurobiology at the Bulgarian Academy of Sciences, University of Chemical Technology and Metallurgy, ELTA 90 Company.**

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PEPTIDE-BASED DRUG DEVELOPMENT BASED ON THE AUTISM/ALZHEIMER'S-LINKED ACTIVITY-DEPENDENT NEUROPROTECTIVE PROTEIN (ADNP)

Illana Gozes

Elton Laboratory for Molecular Neuroendocrinology, Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Adams Super Center for Brain Studies and Sagol School of Neuroscience, Tel Aviv University, 69978, Tel Aviv, Israel

Two decades ago, we discovered the neuropeptide regulated activity-dependent neuroprotective protein (ADNP) and showed that it is essential for brain formation/function. Germline mutations in ADNP lead to the autistic ADNP syndrome. We now revealed Alzheimer's disease-like tauopathy in a postmortem brain of a 7-year-old child with ADNP syndrome (1) and somatic ADNP mutations possibly driving tauopathy in Alzheimer's disease (AD) (2). We further discovered an association between ADNP and sirtuin 1 (SIRT1), a promoter of healthy longevity preventing AD dementia (3). NAP, an eight amino acid peptide (NAPVSIPQ), fragment of ADNP, contains a microtubule end binding protein (EB1/EB3) binding site (SxIP), enhancing ADNP/Tau/microtubule association, and protecting against tauopathy (1, 2). NAP also enhances SIRT1/Tau/microtubule/EB1 interaction in human neural progenitor cells (3). Interestingly, the active site, SKIP and selected analogues, partly maintain microtubule protective properties of NAP (4, 5). Recently, we extended SKIP-microtubule/cellular protection to Parkinson's disease (PD) models of the microtubule disrupting drug (rotenone) toxicity (6). With rotenone toxicity linked with increased dopaminergic cell death hallmarking PD, we used the rat model of 6-hydroxydopamine (6-OHDA) showing SKIP pretreatment protection against nigral dopaminergic cell degeneration and improved motor behavior (6). This was coupled to ADNP/SIR1 dysregulation in the postmortem substantia nigra of PD brains (3). With converging pathologies on ADNP/microtubule dysfunction coupled with tauopathy, we now aim to further develop these peptide protective drug candidates.

References:

1. Grigg, Ivashko-Pachima, Hait…Gozes.Transl Psychiatry.2020;10(1):228.
2. Ivashko-Pachima, Hadar…Gozes. Mol Psychiatry. 2021;26(5):1619-1633.
3. Hadar, Kapitansky...Gozes. Mol Psychiatry. 2021, May, EPUB
4. Sragovich, Amram, Yeheskel, Gozes. Front Cell Neurosci. 2020;13:589.
5. Ivashko-Pachima, Gozes. Front Cell Neurosci. 2021;15:687301.
6. Ivashko-Pachima, Seroogy, Sharabi, Gozes. J Mol Neurosci. 2021 July, EPUB

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UNNATURAL AMINO ACIDS AS A PRIVILEGED CLASS OF BUILDING BLOCKS IN DRUG DESIGN

Tamara Pajpanova

Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev Str, bl 21, 1113 Sofia, Bulgaria

Amino acids are a privileged class of building blocks in drug design. Synthesis of new unusual amino acids has always been in focus of our research for more than 20 years ago.

The numerous proposed examples of unnatural amino acids could include applications as: anti-cancer drugs; antibiotics that would be able to thwart bacterial resistance; drugs that inhibit the formation of amyloid aggregates such as those seen in Alzheimer's, Parkinson's and other diseases; radiopharmaceuticals and components of pharmaceuticals.

We have been focused also, on the roles of non-protein amino acids in modulating stability, potency, permeability and oral bioavailability of the peptides.

We are presenting the predicament faced in the peptide therapeutics and how non-proteinogenic amino acids incorporation can play a role in improving peptide pharmacokinetic properties.

A NEW BIOINSPIRED PEPTIDE WITH POTENTIAL SELF-ASSEMBLY PROPERTIES

Vasile Robert Gradinaru[1], Cosmin Stefan Mocanu[1], Brindusa Alina Petre[1,2], Laura Darie Ion[1], Gabi Drochioiu[1], Mihaela Homocianu[3], Loredana Elena Nita[3]

[1]Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi, 11 Carol I av, Iasi 700506, Romania.
[2]TRANSCEND Research Center, Regional Institute of Oncology, General Henri Mathias, No.2-4, Iasi, 700483, Romania.
[3]Petru Poni Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alee, Iasi 700487, Romania

Peptides are important biomolecules that can afford our understanding of self-assembly and can be used in various applications (1, 2). Here, a new octapeptide is described. This peptide was synthesized by solid-phase synthesis, using Fmoc/t-Bu strategy, purified by HPLC and analyzed by mass spectrometry, UV spectroscopy and fluorescence. Our DLS study suggests that peptide forms nanostructures and the particle size is influenced by concentration and temperature. The ability of self-assembly in solution was demonstrated using FAD and Congo Red as extrinsic fluorescence reporters. The peptide tendency for self-association was also predicted by dynamic simulation studies. Overall, these results open a new research perspective in designing of novel biomaterials with self-assembled properties.

References:

1. S. Eskandari, T. Guerin, I. Toth and R. J. Stephenson, Recent advances in self-assembled peptides: Implications for targeted drug delivery and vaccine engineering, *Adv. Drug Deliv. Rev.*, 2017, 110–111, 169–187.
2. D. O. Sohutskay, T. J. Puls and S. L. Voytik-Harbin, Collagen Self-assembly: Biophysics and Biosigignaling for Advanced Tissue Generation. In: Zhang Y. (eds) Multi-scale Extracellular Matrix Mechanics and Mechanobiology. Studies in Mechanobiology, Tissue Engineering and Biomaterials, vol 23. Springer, Cham, 203–245, 2020.

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EXERCISE INDUCED ALTERATIONS OF CIRCULATORY MRNA LEVELS OF HEAT SHOCK PROTEINS

Selma Arzu Vardar

Department of Physiology, Medical Faculty, Trakya University, Edirne, Turkey

Exercise improves physical performance and may induce positive cardiovascular and metabolic effects on systolic, diastolic blood pressure and insulin sensitivity. Recent studies have been shown that high intensity exercise may have different effects than moderate or low intensity of exercise. In addition to the apoptotic and inflammatory cell signals, high intensity exercise induces the gene expression of heat shock proteins (HSPs) in various cellular compartments including mitochondria and endoplasmic reticulum in mussels or other tissues. Exercise induced changes in gene expression levels of HSPs were also determined in blood.

HSPs as a group of proteins, are described as molecular chaperones to maintain homeostasis in the cells, in tissues and all organism by folding cellular proteins. HSPs may also play a different role into circulation as a byproduct of cellular damage. The alterations of gene expression of HSPs have been shown in plasma following exercise.

In generally, HSP70, HSP72 and HSP90 have been investigated following different intensity of exercise and shows complexity due to differences of exercise intensity and duration. HSP70 has been demonstrated as the most abundant form of HSP family for regulation of exercise in skeletal muscles. HSP60 is mainly expressed in mitochondrial matrix and may be related with metabolic adaptation to regular physical exercise. HSP27 may play repairing role against exercise induced DNA damage and may have beneficial effects for health. We have investigated strenuous exercise induced gene expression levels of HSP's in blood. According to our findings, exercise may increase of gene expression level of HSP27, HSP60 and HSP70 in circulation whereas no alteration has been shown in HSP90 gene expression in healthy subjects.

Training status of the subjects may be another factor on the circulatory gene expression of HSP's. According to our recent study, gene expressions of HSP's levels in circulation may change with physical activity levels of the subjects. Further studies are needed to explore importance of exercise induced changes of HSP's in blood.

IRISIN AND ITS PHYSIOPATHOLOGICAL ROLE IN THE KIDNEY

Nurettin Aydogdu

Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

Irisin is a polypeptide consisting of 112 amino acids discovered in 2012. Irisin is first synthesized in skeletal muscle cells after exercise. Other cell types have also been shown to synthesize and secrete irisin. It is reported that irisin has protective and therapeutic effects in many different tissues and organs, including kidney, adipose tissue, nervous and skeletal systems.

Recent studies have revealed that irisin participates in the pathophysiological process of various diseases by inhibiting apoptosis, reducing inflammation and improving oxidative stress. It has been reported that serum irisin levels are decreased in patients with chronic kidney injury and type 2 diabetes. In contrast, serum Irisin levels have been shown to be elevated in patients with chronic kidney disease or end-stage renal disease and in long-term dialysis patients. In mice, administration of recombinant irisin has been shown to reduce kidney damage and fibrosis and improve kidney function.

In the studies we carried out in different experimental models in our laboratory; 1) No effect of prolonged high protein diet on kidney irisin expression was observed. 2) in the experimental model of hypertension (3 weeks), chronic irisin treatment at physiological dose did not lower blood pressure and irisin levels were unchanged. 3) In another hypertension model (6 weeks), serum irisin levels were observed to decrease significantly. 4) In the experimental myoglobinuric acute kidney injury model of irisin, there was a decrease in urinary irisin levels and renal tissue irisin expression, while serum irisin levels increased over time. 5) Urine irisin levels were negatively correlated with creatinine clearance in the renal ischemia reperfusion model; showed a positive correlation with fractional sodium and potassium excretion.

Our laboratory findings suggest that irisin and its receptor may be associated with local regulation of kidney function and the pathophysiology of kidney injury. We also think that irisin can be used as a biomarker in the pathogenesis of kidney damage. We believe that more studies are needed to understand the mechanisms underlying the role of irisin in the pathophysiology of kidney injury and the relationship between glomerular and tubular dysfunction.

FOLDED OLIGOMERS FOR CELLULAR UPTAKE AND DRUG DELIVERY

Lubomir L. Vezenkov, Baptiste Legrand, Vincent Martin, Nadir Bettache1, Jean Martinez1, Nabila Laroui, Matthieu Simon, Muriel Amblard

ENSCM/IBMM, 26-234 Av. du Professeur Emile Jeanbrau, 34090 Montpellier

Many potential drugs are ineffective because of their inability to cross certain biological membranes, such as the lipid bilayer or the blood-brain barrier. Once inside the cell, those compounds often have to find a local address, also known as cell compartment, but unfortunately often they will lose their way and find themselves trapped in the wrong cell organelles. Cell penetrating peptides (CPP) gave to a certain extent an answer to this problem by delivering a large array of bioactive molecules. While potent, those compounds share some of the general peptide drawbacks like low bioavailability, enzymatic instability and in the case of polycationic compounds sometimes toxicity. In our group, we sought to develop an alternative class of compounds based on structurally organized oligomers also known as foldamers or stapled peptides. We designed and synthesized a first generation of vectors for cellular penetration composed of dipeptide mimetics that had the particularity to be non-charged and were used to deliver dies[1], a mass spectrometry tag[2] and an anti-cancer drug[3,4] to the endo-lysosomal compartment. Next, we have developed a second generation of amphipathic vectors that adopt a ribbon structure, possess low cationic content and target the cytosol compartment. These oligomers were used to deliver a die and a pro-apoptotic peptide inside MDA-MB-231 cancer cells.[5,6] This class of compounds exhibited remarkable enzymatic degradation resistance and very high cellular uptake compared to the reference CPP Penetratin. Recently, we have additionally developed short, highly organized stapled peptides that were able to encapsulate and transport SiRNA molecules with high efficiency.[7]

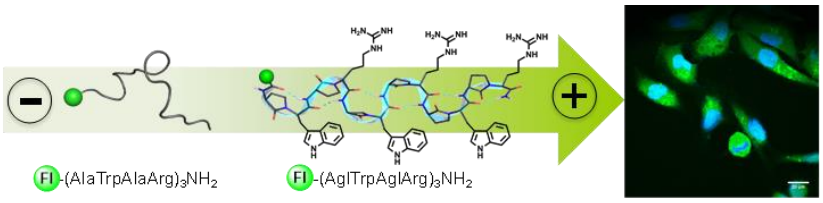

Figure 1. Figure caption example

References:

1. Vezenkov LL et al. *Bioconjug. Chem.* (2010); **21**: 1850–1854; DOI:10.1021/bc1002086.
2. Paramelle et al. *Angew. Chem. Int. Ed.* (2010); **49**: 8240–8243; DOI:10.1002/anie.201003347.
3. Vezenkov LL et al. *J. Controlled Release* (2013); **171**: 251–257; DOI:10.1016/j.jconrel.2013.07.017.
4. Vezenkov LL et al. *ChemMedChem* (2016); **11**: 302–308; DOI:10.1002/cmdc.201500457.
5. Martin V et al *Angew. Chem. Int. Ed.* (2015); **54**: 13966–13970; DOI:10.1002/anie.201506955.
6. Vezenkov LL et al. *ChemBioChem* (2017); DOI:10.1002/cbic.201700455.
7. Simon M et al. Nanomaterials (2020) https://doi.org/10.3390/nano10122334

ANALYSIS OF THE DOCKING RESULTS OF SOME SELECTIVE MOR AND DOR LIGANDS

Tatyana Dzimbova[1,2], Fatima Sapundzhi[1], Nevena Pencheva[1], Peter Milanov[1,3]

[1]South-West University "Neofit Rilski", Blagoevgrad, Bulgaria
[2]Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[3]Institute of Mathematics and Informatics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Endogenous opioid peptides are small molecules that are naturally produced in the central nervous system (CNS) and in various glands throughout the body, such as the pituitary and adrenal glands. These peptides produce the same effects as the chemicals known as classic alkaloid opiates, which include morphine and heroin. Endogenous opioid peptides function both as hormones and as neuromodulators. A given opioid peptide can interact with more than one type of opioid receptor. The binding of opioid peptides to these receptors initiates a series of biochemical events that culminate in various effects, including analgesia and euphoria[1]. The aim of the present study was to analyze the results of docking of ligands with MOR and DOR to identify the key elements required for selectivity. 80 ligands with different chemical structure were used in the study. Many of them have been synthesized and biologically tested by our colleagues. The other part are compounds known in the literature. The compounds were modeled with Avogadro software, the structure of both receptors was obtained from RCSB (PDB id: 4dkl – MOR and 4ej4 – DOR), and docking was performed with GOLD 5.2 software. Despite the great similarity between the structures of the two receptors, the binding of the ligands to them is different. The analysis of the obtained ligand-receptor complexes makes it possible to determine the key structural elements associated with the manifestation of specificity with respect to one or the other receptor. These results will assist in the design of new compounds with potential MOR or DOR agonistic or antagonistic effects.

Reference:

1. Froehlich JC (1997) Opioid peptides. *Alcohol Health & Research World* 21(2), 132-136.

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APPETITE-RELATED PEPTIDES IN FRUCTOSE-DRINKING RAT MODELS

Petya Hadzhibozheva[1], L. Pashova-Stoyanova[1], Zhivka Tsokeva[1], Maria Ganeva[1], Krasimira Nancheva[2], Galina Ilieva[1], V. Nanchev[3], Anna Tolekova[4], Tsvetelin Georgiev[1]

[1]Department of Physiology, Pathophysiology and Pharmacology, Faculty of Medicine, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria
[2]University Hospital "Stoyan Kirkovitch", 6000 Stara Zagora, Bulgaria
[3]Regional Health Inspectorate, Stara Zagora 6000, Bulgaria
[4]Medical College, Stara Zagora 6000, Bulgaria

The aim of this research was to examine the effects of drinking fructose on the appetite – related hormone plasma levels (ghrelin, leptin, insulin) in male and female rats.

Mature Wistar rats were randomly divided as follows: two control groups - male (MC) and female (FC), received tap water to drink; two fructose-drinking groups - male (MF) and female (FF), received 15% fructose solution. The study duration was 11 weeks and during this period body parameters were monitored weekly. At the end of the experiment, ghrelin, leptin and insulin levels as well as lipid and glucose profile were assessed.

Both MF and FF groups had elevated blood glucose and triglyceride levels at the end of the study. Significant differences in the weight gain between each control and the relevant fructose drinking-group was not found. In fructose-drinking groups, plasma insulin, ghrelin and leptin concentrations were elevated, but in the MF group only the leptin levels were significantly higher compared to the MC. Regarding FF group, all the three examined appetite-related hormones were with the highest concentrations among all the groups.

Drinking of 15% fructose solution for 11 weeks affects the peripheral appetite-related peptides, blood glucose profile and lipids. These effects seem to precede the development of overweight in the rats. The elevation of the plasma insulin, ghrelin and leptin concentrations in fructose-drinking rat models is likely to be a gender-dependent. Probably sex hormones are involved in the change of the appetite-regulation signals and are of importance for the degree of development of the metabolic disorders observed in long-term fructose overconsumption.

STRUCTURE-ACTITIVTY RELATIONSHIP OF MULTITARGET DIRECTED GALANTHAMINE-PEPTIDE DERIVATIVES DESIGNED FOR THE TREATMENT OF ALZHEIMER'S DESEASE

Alessandro Pesaresi[1], Doriano Lamba[1,2], Lyubomir T. Vezenkov[3], Daniela S. Tsekova[3]

[1]Istituto di Cristallografia - CNR, Trieste Outstation, Trieste, Italy
[2]Istituto Nazionale Biostrutture e Biosistemi, Consorzio Interuniversitario, Rome, Italy
[3]Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Alzheimer's disease (AD) is a neurodegenerative disorder with multifactorial etiopathology in which both genetic and endogenous factors play a role.

The two major hallmark of Alzheimer's disease (AD) are the imparement of the cholinergic neuronal network and the extracellular deposition of the β-amyloid peptide (Aβ). Aβ is a 39−42 amino acid peptide generated from the amyloid precursor protein (APP) via sequential cleavage by two enzymes, β-secretase and γ-secretase. Although several research strategies have been envisaged in recent decades, increasing cholinergic neurotransmission by inhibiting the enzyme acetylcholinesterase (AChE) and the inhibition of γ/β-secretase still represents the main treatment options for AD.

The complex interconnection of the molecular events underpinning AD implies that the success of a therapeutic approach is likely to depend on the simultaneous modulation of more than one pathological target. This led to a new paradigm in drug discovery for AD, namely the development of multifunctional molecules as multitarget directed ligands (MTDLs), (1).

Here we report on the characterization of the inhibition of AChE by a series of MTDL derived from the combination of galanthamine, a well known AChE inhibitor, with 7 different tetra/penta peptides endowed with β-secretase inhibitory activity (2, 3). The integration of kinetic inhibition data with automated docking simulations provided insights into the mechanism of inhibition and allowed to outline a detailed structure-activity relationship.

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STUDY OF NOVEL ANTIMICROBIAL PEPTIDES AND GLYCOPEPTIDES IN *CORNU ASPERSUM* MUCUS

Lyudmila Velkova, Aleksandar Dolashki, Ventseslav Atanasov, Dimitar Kaynarov, Asya Daskalova, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

The discovery of new effective antibacterial agents has become a top priority in the last decades. The mucus from the garden snails *Cornu aspersum* is complex, multi-component mixtures including different biochemical active substances. Previous study has shown that peptide fractions from *C. aspersum* mucus possess strong antimicrobial activity. To explain the observed antimicrobial effects, the peptides and glycopeptides in active fractions were purified by RP-HPLC. All fractions were tested by orcinol/H_2SO_4 to identified glycopeptides. The primary structures of novel antimicrobial peptides were identified by *de novo* sequencing of tandem mass spectrometry. Most of them contain high level of glycine, leucine, proline, tryptophan and valine residues - typical for peptides with antimicrobial activity. The peptides were characterized by ExPASy ProtParam tool.

Several *N*-glycopeptides from high-mannose and complex type in fraction with MW below 5 kDa were determined by their amino acid sequences and carbohydrate structures using Q-trap and nanoflow - LC/MS/MS analyses. Some of them contain terminal MeHex residues and modification to inner core by Xyl and/or Fuc residues.

To identify the carbohydrate structure of peptides in fraction with MW below 10 kDa, the glycans were removed after digestion with the specific endoglycosidase PNGase F and analyzed by MALDI-TOF/TOF-MS and Q-trap MS/MS. Using this approach, 15 novel *N*-linked oligosaccharide structures were determinate. The identified oligosaccharide structures reveal a complex *N*-glycan pattern combining typical structural features of different higher organisms (mammals, plants, insects, nematodes, trematodes).

Important information on the structures of bioactive peptides from *C. aspersum* and their potential biomedical applications has been obtained.

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SYNTHESIS, ANTICANCER AND ANTIMICROBIAL ACTIVITY OF SHORTENED ANALOGUES OF $(KLAKLAK)_2$

Sirine Jaber[1], Emilia Naydenova[1], Tsvetelina Angelova[1], Veronica Nemska[1], Ivan Iliev[2], Inna Solikovska[2], Nelly Georgieva[1], Dancho Danalev[1]

[1]Department of Organic Chemistry, University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd. 1756 Sofia, Bulgaria

[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 25, 1113 Sofia, Bulgaria

$(KLAKLAK)_2$ is a synthetic antimicrobial peptide with antitumor properties (1-3). Herein, we repost the synthesis of C-terminal amide analogues of single moiety of $(KLAKLAK)_2$. All newly synthesized peptides containing unnatural amino acids beta-Ala in the place of Ala and Leu was replaced by nor-Leu. Bioconjugates are biologically active molecules which combine in their structure two active fragments. The main idea of this conjugation is to find synergetic effect of both parts of hybrid molecule but it is possible that two combined moieties act on different targets in human organism. Cytotoxicity, antiproliferative effect and antimicrobial activity of newly synthesized structures were studied *in vitro* against a panel of cell lines and model G+, G- microorganism and fungi, respectively. The obtained results for anticancer activity reveal significant selective index for substances with common chemical structure KLβAKLβAK-NH_2. The obtained results reveal that the introducing of second pharmacophore 1,8-naphthalimideGly and caffeic acid increase the cytotoxicity and antiproliferative activity of the peptides but not their selectivity. Only two compounds KLAKLAK-NH_2 and 1,8-naphthalimideGKnLAKnLAK-NH_2 show moderate activity against *Escherichia coli K12* at low concentration of 20μM.

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ANTIBACTERIAL EFFECT OF TWO PEPTIDE/PROTEIN FRACTIONS FROM SNAIL MUCUS – FLUORESCENT AND DIGITAL IMAGE ANALYSIS

Mihaela Belouhova[1], Yana Topalova[1], Elmira Daskalova[1], Lyudmila Velkova[2], Pavlina Dolashka[2]

[1]Department of General and Applied Hydrobiology, Faculty of Biology, Sofia University St. Kliment Ohridski, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

[2]Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

The antibacterial effect usually refers to bacteria's elimination by the action of an antibacterial agent. However, the naturally found compounds, such as the antibacterial peptides, usually show more mild action against bacteria. This could give an advantage to the human organism and could contribute to the elimination of real infections. The latter effect is very difficult for estimation by the standard plate count techniques. That is why the aim of this study was to estimate the antibacterial effect of two peptide fractions by applying fluorescent analysis and digital image analysis. The peptide fractions were isolated from snail mucus from *Cornu aspersum* and they had MW below 10 kDa and above 30 kDa. They were applied in concentrations of 1%, 5%, and 10% for 6 hours, and 50% for 1 hour. A reference strain of *Escherichia coli* was used as model microorganisms. The applied approach showed inhibition of the metabolic activity up to 21% by the lower concentrations of the fractions. The application of 50% of the peptide/protein fractions led to inhibition of the metabolic activity with up to 19%. Also, the bacteria decreased their size by 35% for the two fractions. When the lower concentrations were applied more complex effects were found – a decrease of the bacteria's size (up to 24%) in the presence of 1% and 5% of the biologically active compounds; and an increase of the size with up to 42% when 10% was applied. This was most probably related to the inhibition of the bacteria's ability to separate during cell division. The part of the live bacteria after incubation with 50 % of the biologically active substances was lower with 35% for the two fractions. The results highlighted the increased bacterial mortality when higher concentrations of the natural peptide/protein fractions were used and the inhibition of the cells functioning when lower concentrations were applied.

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INVESTIGATION OF CONFORMATIONAL CHANGES IN BIOACTIVE FRACTIONS AND PEPTIDES FROM MUCUS OF CORNU ASPERSUM BY FLUORESCENCE SPECTROSCOPY

Asya Daskalova, Lyudmila Velkova, Aleksandar Dolashki, Dimitar Kaynarov, Ventseslav Atanasov, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

It is known that the three amino acids phenylalanine, tyrosine and tryptophan are aromatic and therefore all fluorescent. Fluorescence spectroscopy is a crucial tool in biochemical research because of its high sensitivity and non-invasiveness.

The conformational stability of both peptide fractions with molecular weight below 3 kDa and below 10 kDa, isolated from garden snail mucus, was tested by fluorescence spectroscopy in the presence of two denaturants with increasing concentrations by excitation at 295 nm. Moreover, the primary structures of 40 new mucus peptides of *Cornu aspersum* garden snail were recently analyzed through mass spectrometry. Two of these peptides MLGGGWNPPK (MW 1055.52 Da) and LLFGVAWQNGLRQQ (MW 1628.88 Da) were synthesized and analyses for conformational changes in their structures were performed through increasing concentrations of urea and guanidine hydrochloride (Gnd. HCl).

One λ_{max} at 350 nm was identified in the fluorescence specter of peptide MLGGGWNPPK after excitation at 295 nm, which corresponds to tryptophan residues. In contrast, the spectra of the second peptide showed one λ_{max} at 328 nm and a new maximum at 350 nm due to the increasing concentration of (Gnd. HCl). The fluorescence spectra of both peptide fractions (MW< 3 kDa and 10 kDa) showed maximum at 336 nm, typically for "buried" tryptophan side chains after excitation at 295 nm.

The observed differences in unfolding of the peptides and the peptide fractions are due to the fact that Gnd.HCL affects hydrophobic and ionic interactions simultaneously.

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TREATMENT OF WOUNDS WITH NATURAL SUBSTANCES

Momchil Kermedchiev[1,2], Konstantin Angelov[2], Alexander Dolashki[2], Lyudmila Velkova[2], Pavlina Dolashka[2]

[1]Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria
[2]UMHAT "Alexandrovska", Emergency Department and Surgery, Sofia, Bulgaria

Some wounds do not heal normally and can be considered difficult to heal and chronic wounds. Therefore, they need to be treated with a new methodology and appropriate and timely measures taken to correct the root cause of difficult-to-heal wounds. Knowledge of the therapeutic effect of snail mucus and various types of medicinal herbs in the treatment of wounds is known.

Patients with difficult-to-heal and chronic wounds of different ages and genesis (diabetic gangrene, chronic venous insufficiency, chronic arterial insufficiency and pressure ulcers), as well as with various comorbidities, were selected prospectively of different sex and age.

The same future new protocol, a new approach to antiseptic wound treatment, a new pace and type of dressings, a treatment plan, training of the patient and relatives for self-help and dressings are applied. Several parameters were analyzed such as pH date, bacterial flora, local wound condition and inflammatory process, pain level, healing rate. The new approach used in the treatment of difficult-to-heal wounds with snail mucus from Helix aspersa and herbal essential oils shows promising results.

There is a significant reduction in wound healing time, rapid and effective reduction of the local inflammatory process and chronic pain. The obtained results show that the healing of such wounds is promoted not only by limiting the bacterial infection, but also by stimulating the growth of the tissues and providing them with a suitable local alkaline-acidic, moist and nutritious environment. Mass spectrometric analysis of mucus and plant extracts showed the presence of peptides and glycopeptides with proven antibacterial and regenerative effect. Early and regular application of our proposed new approach to treatment will prevent amputation of the legs and will serve as the main therapy for the treatment of chronic wounds.

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NOVEL PEPTIDES IN THE HEMOLYMPH OF CORNU ASPERSUM SNAIL IDENTIFIED BY MASS SPECTROMETRY

Aleksandar Dolashki, Lyudmila Velkova, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

The discovery and characterization of new biologically active substances from natural sources has been extremely relevant in recent years.

The aim of this study is to isolate and identify new peptides from the hemolymph of the garden snail *Cornu aspersum*. After isolation by ultrafiltration, the fraction with Mw <3 kDa from *C. aspersum* hemolymph was purified by reverse phase high performance liquid chromatography (RP-HPLC) and analyzed by mass spectrometry.

The molecular weights of the peptides were determined by MALDI-MS analyzes. MALDI-MS/MS was used to identify the primary structures of the peptides. Some of them were confirmed by LC-ESI-MS analyzes of LTQ Orbitrap XL. The primary structures of 24 novel peptides were characterized by *de novo* sequencing from MS/MS analyses. The structural characterizations of the new peptides such as a grand average of hydropathicity (GRAVY), isoelectric point (pI) and a net charge were determined using their amino acid sequences (AAS) by the ExPASy ProtParam tool. In general, peptide structures, containing between 7-11 amino acid residues, mainly with a hydrophobic surface predominated. It was observed high level of Val, Pro, Lys, Asn, Leu/Ile, Met, His, Phe, Trp and Tyr residues, which are typical for peptides with antimicrobial activity and antioxidant properties. The structural characterization of new peptides and the alignment of their AAS through the CAMPSing database reveal high homology with the structures of known antimicrobial peptides.

The obtained results are the basis for further investigations on bioactive compounds from *C. apersum* hemolymph and for the development of new natural products with potential biomedical applications.

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COMPARATIVELY STUDY OF METABOLITES PROFILING OF *HELIX ASPERSA* MUCUS, *HELIX LUCORUM* HEMOLYMPH AND *RAPANA VENOSA* HEMOLYMPH USING ^{1}H NMR AND MASS SPECTROMETRY

Nikolay G. Vassilev, Svetlana Simova, Miroslav Dangalov, Lyudmila Velkova, Venceslav Atanassov, Aleksandar Dolashki, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

Metabolic profiling based on ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy was applied with the aim to investigate the functional role of the metabolites in lyophilized mucus from garden snail *Helix aspersa*. Twenty metabolites were unambiguous identified by ^{1}H, 1D TOCSY, 2D J-resolved, 2D COSY and 2D HSQC NMR spectra with water suppression. The developed protocol for the determination and assignment of metabolites in mucus from *H. aspersa* by NMR spectroscopy (1) was adopted to study the low molecular weight fractions of hemolymph from *H. lucorum* (<1kD and <3kD) and fourteen metabolites were unambiguously identified (2). The same protocal was applied to the low molecular weight fractions of hemolymph from *R. venosa* (<3kD) and eleven metabolites were unambiguously identified.

In the all studies metabolites with known antioxidant, antibacterial and antimicrobial activity have been detected. Some of them were confirmed by mass spectrometric analysis. The primary structure of several peptides was identified in low molecular weight fractions (Mw<1 kDa) by tandem mass spectrometry.

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IDENTIFICATION OF NOVEL GLYCOPEPTIDES IDENTIFIED IN THE MUCUS OF GARDANE SNAIL *CORNU ASPERSUM* BY MASS SPECTROMETRY

Dimitar Kaynarov, Lyudmila Velkova, Aleksandar Dolashki, Asya Daskalova, Ventseslav Atanasov, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

The snail mucus is a complex mixture of bioactive compounds with is known for its healing and stimulating properties. Previous study has shown that peptide fractions from *Cornu aspersum* mucus, containing glycopeptides, possess strong antimicrobial activity including multidrug-resistant bacteria. Several *N*-glycopeptides from mucus fraction with MW<5 kDa were determined by their amino acid sequences and carbohydrate structures using Q-trap system and nanoflow - LC/MS/MS analyses.

With aim to identify the carbohydrate structure of peptides in mucus fraction with MW <10 kDa, the glycans were removed after digestion with the specific endoglycosidase PNGase F and analyzed by MALDI-TOF/TOF-MS and Q-trap MS/MS. The results showed some of them are high-mannose and complex type, containing terminal MeHex residue and modification to inner core by xylose. β(1,2)-Xylose linked to the core mannose is a highly immunogenic epitope for mammalian species. Moreover, structures with two types of Fuc-linked residues were identified: (α1-3) Fuc-linked to an internal GlcNAc residue and (α1-6) Fuc-linked to the terminal GlcNAc-residue of the pentasaccharide core. Using this approach, 17 novel *N*-linked oligosaccharide structures were determinate. The identified carbohydrate structures reveal a complex *N*-glycan pattern combining typical structural features of different higher organisms.

The presented results may be considered as basic information for further investigations on bioactive compounds from the mucus of garden snail *C. aspersum*.

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DETERMINATION OF THE CONFORMATIONAL STABILITY OF PEPTIDES FROM *HELIX ASPERSA* SNAIL BY CIRCULAR DICHROISM

Aleksandar Dolashki, Ventseslav Atanasov, Lyudmila Velkova, Asya Daskalova, Dimitar Kaynarov, Pavlina Dolashka

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

The various biomedical applications of the mucus from *Cornu aspersum*, associated with their antimicrobial activity and regenerative properties promoted us to perform structural studies by UV circular dichroism (CD).

The conformational changes of two mucus fractions with MW <3 kDa and MW <10 kDa as well as of several peptides (purified by rechromatography of RP-HPLC), were analyzed in a wide temperature range between 25-85ºC at pH 7.5 in 50 mM Tris buffer (pH 7.5) via their CD spectra, recorded with a J-720 spectropolarimeter (Jasco, Tokyo, Japan) in the UV range between 200 nm and 250 nm.

A characteristic feature is the T-induced changes within a wide temperature interval 25°C-85°C, a drastic decrease in negative ellipticity ($[\theta]_\lambda$) was observed at temperatures above 40°C. The results showed that the fraction with MW<10 kDa is higher thermal stability (Tm=59.6ºC) than fraction <3kDa (Tm=48.6ºC). The relatively small changes of initial $[\theta]_{222}$ and determined Tm=66.8ºC indicated that peptide MLGGGWNPPK is more stable than other peptides: MGVGAVWNGHK (Tm=38.5ºC) NLVGGLSGGRGGAPGG(Tm=55.2ºC) and LLFGVAWQNGLRQQ (Tm=51.8ºC).

The unfolding of native mucus fractions (with MW <10 kDa and MW <3 kDa) in water solutions in the presence of two denaturants (urea and guanidine hydrochloride) were investigated. The free energy of stabilization in water ($\Delta G_D H_2O$) for fraction with MW <10 kDa was calculated in the range of 11.3-11.6 kJ mol^{-1} while for the fraction <3 kDa, the calculated $\Delta G_D H_2O$ is of a lower value: 7.8-8.3 kJ mol^{-1}

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CONFORMATION ANALYSIS OF MODIFIED *HELIX LUCORUM* HEMOCYANIN

Yuliana Raynova, Denitsa Yancheva, Svetla Todinova, Maya Guncheva, Krassimira Idakieva

Institute of Organic Chemistry with Center of Phytochemistry, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

Mollusk hemocyanins (Hcs) are large oxygen-carrier glycoproteins, present in the hemolymph of some mollusks, which are widely used in biomedicine and biotechnology (1). Mollusk Hcs have a carbohydrate content of 2–9 % (w/w), with mannose being the major monosaccharide found in these structures.

Aiming to elucidate the role of the sugar moieties for the conformational stability of *Helix lucorum* hemocyanin (HlH) we modified HlH with sodium periodate.

Native HlH was purified from the hemolymph, collected from garden snails *H. lucorum*, by ultracentrifugation and subsequent gel filtration chromatography. Oxidized carbohydrate chains of HlH give reactive aldehydes, which are involved in the formation of Schiff bases with the free amino groups of the protein e.g. the ε-amino groups of lysine and the amino terminus of the protein. The native and oxidized HlH (oxy-HlH) were characterized by electrophoresis, spectroscopic methods, and differential scanning calorimetry (DSC).

SDS-PAGE analysis showed differences in the mobility pattern between the native and the periodate-treated HlH. ATR-FTIR spectroscopy was used to assess the differences in the secondary structures of the native HlH and oxy-HlH. Rearrangement in the Hc molecule, decrease in α-helices and coiled structures in favour of the β-structures were observed in oxy-HlH in comparison to the native HlH. The DSC curve of the oxy-HlH is characterized with an asymmetric shape, which is an indication of the existence of more than one structural unit in the analyzed sample. The modification of the HlH did not result in a significant change in the position of the endothermic maxima. We assessed the effect of the native and oxy- HlH on the proliferation of human cervical cancer cells (Hela cells). HeLa cells treated for 48 h with HlH and oxy-HlH at a concentration of 50 μg/mL showed reduced by 45 and 56% cell viability, respectively.

This study reveals that sugar moieties in the HlH molecules predetermines the stability, catalytic and biological activity of HlH.

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SYNTHESIS AND CHARACTERIZATION OF COPPER COMPLEXES WITH PEPTIDE LIGAND AS POTENTIAL ANTICANCER AGENTS

Veronika Karadjova, Dessislava Borisova, SpaskaYaneva, Emilia Naydenova, Trayana Dolchinkova, Vladislava Ivanova, Dancho Danalev

University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria

Many metals play key role in different physiological processes in human organism. Some of them as Ni, Co, Fe, Cu are cofactor of enzymes, other as Ca participate in blood coagulation processes and another like magnesium play important role for heart, ATP synthesis, etc. There are metals like Ag and Au with proven antibacterialproperties,Pt and Cu are included in the molecules with proven anticancer activity, etc.On the other hand peptides are good candidates to form complex with different metals due to specific functional groups such as COOH, NH_2, SH of amino acids in their composition. Thus the formed complexes often have better pharmacokinetic profile including better hydrolytic stability at different pH and bioavailability of the carrier molecule. Copper is a toxic metal with well-known antimicrobial activity.However, the mechanism of copper toxic action is not yet fully established. Lately copper isunder profound investigation as an attractive target for inclusion in modern chemotherapeutics for the treatment of cancer cells in combination with second pharmacophore with proven anticancer properties.In a current study we report synthesis and characterization of copper complexes with peptide ligand fluorinated analogues of BIM-23052 with already established antitumor activity. The following main structure Cu(II)-Se(VI)/Cl(I)-peptide ligandof complexes was determined and characterized by FTIR, elemental and ICP analysis as well as Raman spectroscopy. For their synthesis a preliminary prepared selenate compound ($CuSeO_4 \bullet 5H_2O$) was used. It was prepared by means of copper (II) hydroxide carbonate neutralization with diluted solution of selenic acid. The Cu^{2+} ions were introduced from $CuCl_2$. Studies for optimal condition according to solvent solubility of copper salts, concentrations and pH of starting solutions as well as reaction time, structure and stability of complexes will be reported.

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AMANTADINE AND RIMANTADINE ANALOGUES WITH GLYCINE BASED AMINO ACIDS AND SMALL PEPTIDES

Aleksandra Tencheva, Radoslav Chayrov and Ivanka Stankova

Department of Chemistry, South-West University "Neofit Rilski", 2700 Blagoevgrad, Bulgaria

Amantadine is used to treat Parkinson's disease and symptoms such as stiffness or tremors and repetitive uncontrolled muscle movements that may be caused by the use of certain drugs. Both amantadine and rimantadine are used to treat or prevent influenza A in adults and children. Unfortunately, the mutation and hence the acquisition of resistance of such viruses to existing drugs is so fast that the development of new substances with the desired pharmacological properties has become a huge challenge for scientists.

All those facts have prompted a dynamic search for new antiviral drugs, and the creation of prodrugs is a promising alternative in this direction. Herein, we report design and synthesis of series of amantadine and rimantadine derivatives incorporating N-methylglycine, N,N-Dimethylglycine, Di-glycine and Tri-glycine have been synthesized for the first time. Main goal of the new derivatives is to increase amantadine and rimantadine activity.

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IN VIVO AND *IN VITRO* EVALUATION OF THE ANTI-INFLAMMATORY POTENTIAL OF 4-AMINOPYRIDINE DERIVATIVES

Tanya Dimova [1], Rashev P[1], Manchev S[1], Tsekova D[2], Vezenkov L[2]

[1]Institute of Biology and Immunology of Reproduction Acad. "Kiril Bratanov", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[2]University of Chemical Technology and Metallurgy, "Organic chemistry" Department

Three derivatives of 4-aminopyridine (4-AP) were designed and synthesized as follows: 1) substance A (Boc-tert-Leu-Asn-Leu-Ala-4AП), 2) substance B (Boc-Val-Asn-Leu-Ala-4AП) and 3) substance C (Boc-Nva-Asn-Leu-Ale-4AП). In rat model for mild systemic inflammation the anti-inflammatory effect of these three 4-AP derivatives was investigated. The derivatives were tested in two doses for prevention and for treatment of the inflammation. We demonstrated that substance A could significantly reduce anti-BSA antibody levels in both prevented and treated groups in both doses. No effect was observed on the treatment with both substances B and C at any dose. The *in vitro* cytotoxic potential of the investigated compounds was determined using Wehi-164 cells and MTT-based assay and their inhibitory effect on the gelatinolytic activity of MMPs 2 and 9 was evaluated by zymography. The response of Wehi-164 cells to the investigated substances applied at different doses (0.1, 0.2, 0.4 and 0.8 ug/ml) showed no cytotoxic effect of any substance in any dose suggesting that all tested 4-AP derivatives provided viable and metabolically active cells. The enzymes MMP-9 and MMP-2 are responsible for the inflammation and tissue destruction process. Evaluation of the inhibitory effect of 4-AP derivatives on their activity showed a strong inhibitory effect of compounds A and C even at the lowest dose (0.1 ug/ml), while such an effect of compound B was detected only at higher doses (0.2, 0.4 and 0.8 ug/ml). Interestingly, compound C in highest dose (0.8 ug/ml) stimulated MMP-9 activity.

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NEW NATURAL AND SYNTHETIC PEPTIDES AS POSITIVE IMPACT ON HYPERTENSION

Boryana Yakimova[1], Lili Dobreva[2], Proletina Kardaleva[1], Svetla Danova[2] and Ivanka Stoineva[1]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Inhibition of angiotensin - converting enzyme I (ACE I) is a modern therapeutic target in the treatment of hypertension. In recent years, the research concerning natural ACE inhibitors without side effects became an important field. Food proteins, besides their nutritional value, can be a source of bioactive peptides (2-20 amino acid residues) that can modulate several key biological functions. To date, ACE inhibitors derived from food proteins are one of the best-known bioactive peptides. Their biofunctionality, make them a promising alternative to synthetic drugs as well as a good food additives.

Recent studies indicate that milk furnishes a broad range of biologically active compounds that guard neonates and adults against pathogens and illnesses, such as immunoglobulins, antibacterial peptides, antihypertensive peptides, antimicrobial proteins, oligosaccharides, lipids and etc. with considerable potential benefits.

The aim of these research is to study the bioactive peptides derivative from different protein-based dairy products fermented by of selected *Lactobacillus* strains (LAB). The 14 strains isolated from a unique Bulgarian product "katak" from the Balkan area (Bulgaria) were tested. In parallel, the target peptides containing unnatural amino acids were synthesized by Fmoc strategy of SPPS. The newly peptides were purified by HPLC and characterized by UPLC-MS and NMR. The comparative study of ACE I inhibitory activity of the newly synthesized short peptides and isolated nature peptides are in progress now.

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ANTIOXIDANT ACTIVITY OF HEMOLYMPH ISOLATED FROM *RAPANA VENOSA*

Lubomir Petrov[1], Mihail Kachaunov[1], Albena Alexandrova[1]*, Elina Tsvetanova[2], Almira Georgieva[2], Lyudmila Velkova[3], Ventssislav Atanasov[3], Aleksander Dolashki[3], Pavlina Dolashka[3]

[1]National Sports Academy, 21, Acad. Stefan Mladenov, str., 1700 Sofia, BULGARIA
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, 23, Georgi Bonchev str., 1113 Sofia, BULGARIA
[3]Institute of Organic Chemistry, Bulgarian Academy of Sciences, 9, Georgi Bonchev str., 1113 Sofia, BULGARIA

Material and methods: Three fractions of hemolymph with molecular weight (MW) of 1-10 kDa, 10-50 kDa and 30-100 kDa, as well as two hemocyanine subunits RvH1 and RvH , isolated from *R. venosa*, were tested in chemical systems generating reactive oxygen species. Superoxide radicals ($O_2^{\bullet-}$) were generated photochemically and the scavenger effect was estimated as % inhibition of Nitro Blue Tetrazolium (NBT) reduction. The hydroxyl radicals ($^{\bullet}OH$) were generated in Fenton system ($Fe^{2+}+H_20_2$) in presence EDTA and the scavenger effect was estimated as % inhibition of thiobarbituric acid reactive substances (TBARS) formation from deoxyribose.
Results: The highest $O_2^{\bullet-}$ scavenger effect had the fraction with MW 30-100 kDa followed by the fraction with MW 10-50 kDa that demonstrated 50% inhibition of NBT reduction at 28.4 μg/mL and 37.3 μg/mL, respectively. The RvH1 and RvH2 inhibited NBT reduction at about 100 μg/mL. The most powerful $^{\bullet}OH$ scavenger effect showed the fraction with MW 1-10 kDa and 10-50 kDa – above 70% inhibition of TBARS formation at the highest concentration tested (25 mg/mL, final concentration). TheRvH1 and RvH2 had a significantly lower effect - below 50% inhibition at the same concentration.

Conclusions: In this study, we present for the first time an investigation on the antioxidant activities of three fractions of hemolymph with molecular weight of 1-10 kDa, 10-50 kDa and 30-100 kDa, as well as two hemocyanine subunits RvH1 and RvH2, isolated from *Rapana venosa*. The good $O_2^{\bullet-}$ and excellent $^{\bullet}OH$ scavenger effect of hemolymph fraction with MW 10-50 kDa suggests its possible application in pathological conditions with oxidative stress etiology.

Keywords: antioxidant activity, *Rapana venosa*, hemolymph, hemocyanine subunits

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SNAIL (*HELIX ASPERSA*) EXTRACT IMPROVES IMPAIRED SPATIAL LEARNING AND MEMORY IN ALZHEIMER'S TYPE OF DEMENTIA BY REGULATING CREB/BDNF SIGNALING

Maria Lazarova[1], Lyubka Tancheva[1,2], Lyudmila Velkova[3], Alexander Dolashki[3], Diamara Uzunova[1], Petya Gavrilova[1], Krasimira Tasheva[4], Teodora Taseva[4], Yordan K. Hodzhev[5], Atanas G. Atanasov[6,7,8*], Miroslava Stefanova[1], Ventseslav Atanasov[3], Reni Kalfin[1], Pavlina Dolashka[3]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str, bl 23, 1113 Sofia, Bulgaria
[2]Weston Professor of Weizmann Institute of Science, Israel
[3] Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl.9, 1113 Sofia, Bulgaria
[4]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl 21, 1113 Sofia, Bulgaria
[5]National Center for Infectious and Parasitic Diseases - NCIPD, 26 Yanko Sakazov Blvd 1504, Sofia, Bulgaria
[6]Ludwig Boltzmann Institute for Digital Health and Patient Safety, Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria
[7]Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, 05-552 Magdalenka, Poland
[8]Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

Snail mucus from Helix aspersa is a complex, multicomponent mixture containing a number of bioactive substances with different properties. It possess protective potential with multitargeting character and in this way possibility for effectiveness in some neurodegenerative diseases treatment.

The aim of this study was to evaluate the effects of mucus snail extract (SE) from *Helix aspersa* on cognitive impairments induced by scopolamine (Sco). The used for the experiments mucus snail extract contains fresh crude mucus extract enriched with protein mucus fraction with Mw above 20 kDa in relation 1:1. Male Wistar rats (six-eight weeks old) were randomly divided into 4 experimantal groups (n=10): 1) Control group; 2) Sco-treated group; 3) Snail group; 4) Sco+Snail group. Snail extract (0.5 mL/100 g bw) was applied orally for 16 consecutive days - 5 days before and 11 days simultaneously with Sco (2 mg/kg, intraperitonealy). Various behavior tests including the Barnes maze, T-maze and Hole board test were used to evaluate changes in cognitive functions of rats after scopolamine treatment. As a part of molecular mechanism of memory processes the change in acetylcholine (ACh) levels and expression of brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) level in hippocampus were determined. It was found that SE treatment attenuated the scopolamine-induced cognitive impairments in used behavioral tests. SE showed tendency to reverse the Ach levels depressed by Sco and significantly to enhance the expression of CREB/BDNF signaling in the hippocampus.

In conclusion our findings suggest that snail extract exhibits a cognition-enhancing potential and improves the memory impairments in Alzheimer's type of dementia which is mediated by activation of CREB/BDNF signaling in the hippocampus.

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NEUROPROTECTIVE AND ANTIOXIDANT EFFECT OF FRESH SNAIL EXTRACT *(HELIX ASPERSA)* IN RAT'S BRAIN IN ALZHEIMER'S TYPE OF DEMENTIA

Elina Tsvetanova[1], Albena Alexandrova[1,3], Almira Georgieva[1], Lyubka Tancheva[1], Maria Lazarova[1], Reni Kalfin[1], Miroslava Stefanova[1], Alexander Dolashki[2], Lyudmila Velkova[2], Pavlinka Dolashka[2]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Bulgaria
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Bulgaria
[3]National Sports Academy, Bulgaria

Neurodegenerative disorders are characterized by selective neuronal loss, glial activation, inflammatory processes and impaired oxidative metabolism. Reactive oxygen species **(**ROS) cause dysfunction and oxidation of key cellular signaling proteins, also induced the expression of neuropeptide genes. Snail mucus and hemolymph are complex, multicomponent fluids, rich in molecules with different properties.

The aim of the present study was to establish potential neuroprotective and antioxidant effect of snail extract (SE) from *Helix aspersa* in rat's brain in experimental Alzheimer's type dementia. Mucus was collected from snail *Helix aspersa*, enriched with protein mucus fraction with Mw above 20 kDa in relation 1:1. Male Wistar rats weighing 180-200g were divided into 4 groups: control, scopolamine (Sco), snail+Sco and snail. Model was induced via intraperitoneal injections of scop 2 mg for 11 days. Snail+Sco group rats treated for 16 days with SE orally (5 days before, and 11 days simultaneously with Sco), while SE group was treated only with fresh SE. In the beginning and in the end of experiments the animals were subjected to to T-maze test for spatial working memory evaluation. After that rat's brains were removed, separated main structures mostly related to memory - cortex and hippocampus and studied for changes in antioxidant enzyme activities: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

The obtained results of behavioral test significant increased the number of right choices of the animals from snail+Sco vs Sco, accompanied with changes in oxidative status: decreased by Sco SOD activity and increased CAT activity in both structures, also GPx activity was decreased in cortex and increased in hippocamp. SE recovered initial levels only in hippocampus compared with scop group.

Obviously, SE is promising neuroprotective agent with antioxidant properties which deserves further multicomponent investigations on mechanisms involved in its preventing memory effect.

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IN VITRO ANTIMICROBIAL INVESTIGATIONS OF SUBSTITUTED CINNAMIC ACID AMIDES

Maya Chochkova[1], Anna-Maria Hristova[1], Boyka Stoykova[1], Yuhuan Li[2], Iva Tsvetkova[3], Hristo Najdenski[3], Yavor Mitrev[4], Martin Štícha[5]

[1]South-West University ''Neofit Rilski", Blagoevgrad, Bulgaria
[2]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P. R. China
[3]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences
[5]Charles University, Prague, Czech Republic

In this study, different substituted cinnamic acids were coupled with aminoadamantane analogue, memantine to study *in vitro* their preliminary antiviral, antibacterial and antifungal activities. The antiviral screening results indicated that in comparison with clinically used anti-influenza drugs (oseltamivir, ribavirin and amantadine), the group of memantine analogues showed weaker antiviral activity against the influenza strains (A/Wuhan/359/1995 and B/Jinfang/13/1997) tested. Amongst the tested amides, memantine conjugate of (E)-3-(3', 5'-dimethoxyphenyl-4'-hydroxy) propenoic acid occurred to be effective against both strains. Antibacterial and antifungal potentials of the obtained analogues were also investigated *in vitro* against *Staphilococcus aureus* -209 (G+), *Escherichia coli*-WF+ (G-) and the pathogenic fungus - *Candida albicans*- 562. The results revealed that the tested amides are devoid of any inhibitory effect against the tested strains and fungus.

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ANTIPROLIFERATIVE EFFECT OF NOVEL RGD PEPTIDE ANALOGUES

Aneliya Balacheva, Roumyana Detcheva, Tamara Pajpanova

Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev Str, bl 21, 1113 Sofia, Bulgaria

The amino acid sequence L-arginyl-glycyl-L-aspartic acid (RGD) plays the role as minimal sequence in the interaction of many adhesion proteins by binding to certain types of integrin receptors, which are overexpressed on various cancer and endothelial cells. Integrin-binding peptides can be used to transport for cytotoxic molecules to cancer cells. The synthetic RGD peptides can affect adhesion and formation of tumor metastases or directly induce apoptosis by activating caspases. RGD peptides have been the subject of intensive preclinical and clinical studies as antimetastatic agents in the targeted antitumor therapy and for cancer imaging. In this regard, we synthesized a series of model RGD mimetics modified at the N- and C- terminus. We investigated their antiproliferative activity against MCF-7 breast cancer cells after 72 hours of treatment with MTT analysis. We researched the possibility of co-administration of selected peptide mimetics with cisplatin *in vitro* for a potential synergistic cytotoxic effect.

Key words: RGD, peptides, cytotoxicity

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EFFECT OF AMINO ACID-BASED IONIC LIQUIDS ON INSULIN AMYLOID AGGREGATION

Proletina Kardaleva*[,1], Vadimir Vanik[2], Zuzana Gazova[2], Diana Fedunova[2], Denitsa Panteleeva[2], Maya Guncheva[2]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev 9 Str. Sofia 1113, Bulgaria; [2]Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Košice, Slovakia

Aggregation of therapeutic proteins is a serious problem for the pharmaceutical industry. In general, protein aggregates have reduced activity, solubility, and altered immunogenicity. Moreover, *in vitro* aggregation and precipitation hampers the efficiency of biotechnological processes. Numerous studies have shown that some 1, 3-dialkylimidazolium-, alkylammonium- or cholinium-based ionic liquids are able to prevent protein aggregation or stimulate refolding of already aggregated thermally or chemically unfolded proteins. In this research, we investigated the effect of series of ionic liquids (ILs) containing cholinium and 1-ethyl-3-methyl imidazolium cations and non-polar, polar, and charged aminoacid anions: Gly, Thr, Glu, or Lys on stability and aggregation of human insulin. The molar ratio of protein to IL was 1:500. Expectedly, the ILs induce changes in the secondary structure of the protein, which is clearly seen in the deconvoluted in the Amide I region (1700-1600 cm^{-1}) ATR-FTIR spectra of the IL-protein complexes. Interestingly, all tested ILs promote insulin aggregation and the rate of aggregation is from 3.8 to 4.6 times higher than that of the control without ILs. Except for CholGly, we observed a shortening of the lag-time of insulin aggregation in the presence of all tested cholinium-ILs. The insulin fibrils obtained upon heating at pH 1.6 in the presence of 10 mM IL or in IL-free media are morphologically similar as is shown by AFM.

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ANTIOXIDANT PROPERTIES OF A LOW MOLECULAR PEPTIDE FRACTION FROM THE GARDEN SNAIL *HELIX ASPERSA* POSSESSING ANTIFUNGAL ACTIVITY

Ekaterina Krumova, Pavlina Dolashka, Nedelina Kostadinova, Radoslav Abrashev, Jeny Miteva-Staleva, Aleksander Dolashki, Lyudmila Velkova, Vladislava Dishlijska, Boryana Spasova, Maria Angelova

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

Bioactive peptides from snails have been intensively investigated for their antimicrobial, cytotoxic, anti-tumor, and anti-inflammatory activities. However, the mechanisms responsible for these effects are still not clear. It is of interest to investigate the role of antioxidant properties of the natural peptides in their antifungal activity. Our previous studies demonstrated significant fungal growth inhibition by different peptide fractions obtained from the mucus of the garden snail *Helix aspersa*. The fraction with molecular mass between 2 and 20 kDa (Ha/2-20) inhibited the spore germination and biomass formation of the pathogenic fungal strains belonging to the genera *Aspergillus* and *Penicillium*. The aim of this study was to investigate the relationship between antifungal and antioxidant activities of this fraction. The mucus was collected and purified using different membrane technique. The crude extract was separated using Millipore filters by ultrafiltration. The antioxidant properties were evaluated using three test methods, including measurement of the radical scavenging activity on the 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH), total antioxidant activity (ABTS method) and the inhibition of nitro blue tetrazolium (NBT) reduction by photochemically generated superoxide radicals ($^{\bullet}O_2^-$). The results demonstrated that Ha/2-20 possess strong radical scavenging activity. In addition, the cell response of the strains *A. niger* and *P. griseofulfum* against the treatment with this peptide fraction was detected. The fraction H/2-20 induced strain-dependent changes in the oxidative stress biomarkers and antioxidant enzyme activity.

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BIOLOGICAL ACTIVITY OF AMINO ACID DERIVATIVES OF MEMANTINE

Ivanka Stankova[1], Aleksandra Tencheva, Radoslav Chayrov, Tsvetelina Angelova[2], Veronica Nemska[2], Nelly Georgieva[2] and Dancho Danalev[2]

[1]Department of Chemistry, South-West University "Neofit Rilski", 2700 Blagoevgrad, Bulgaria
[2]University of Chemical Technology and Metallurgy, Biotechnology Department, Sofia 1756, Bulgaria

Memantine is adamantane derivative used in medicinal practice. First of all it is well-proven inhibitors of the M2 ion channel of influenza virus and on the other hand it is applied for treatment of patients with Alzheimer's disease. Herein, we report the antimicrobial activity of specifically designed memantine derivatives containing spatially compact (Gly, Ala, β-Ala) and bulky (Val and Phe) amino acids. New compounds were synthesized in a solution using TBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) as a coupling agent. All derivatives were obtained with good yields. Newly synthesized molecules at concentration 100mM were tested for their antimicrobial activity using model strains of Gram-positive (Bacillus subtilis NBIMCC 3562), Gram-negative (Escherichia coli NBIMCC K12 407) microorganisms and fungal strain Candida albicans NBIMCC 74. The active compounds against model G+ microorganism are Val-MEM followed by Ala-MEM and Gly-MEM. All compounds show good activity against model G- microorganism. Introducing both aromatic Phe and bulky amino acid Val leads to good activity against fungi.

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NEUROTENSIN (NT_{8-13}) PEPTIDE MIMETICS: VIRTUAL MULTI-TARGET ACTIVITY ESTIMATION AND STABILITY STUDIES

Iliyan V. Ognyanov[1], Ivan P. Bogdanov[1], Maya G. Georgieva[1], Martina I. Peeva[1], Aneliya A. Balacheva[1], Toni Kühl[2], Tamara I. Pajpanova[1], Diana Imhof[2] and Nikolay T. Tzvetkov[1,*]

[1]Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G. Bonchev str. Bl. 21, Sofia 1113, Bulgaria, e-mail: ntzvetkov@bio21.bas.bg
[2]Pharmaceutical Biochemistry and Bioanalytics, Pharmaceutical Institute, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

Structurally optimized neurotensin (NT) peptide analogs were recently developed as potential pharmacological agents for Parkinson's disease (PD) treatment [1]. Based on their improved pharmacological profile and relatively slow biodegradation combined with good physicochemical properties, two NT-analogues peptidomimetics, have been advanced into an *in vivo* evaluation in a rat PD model [1].

Here we present further explorations within a series of five NT(8-13) analogues with privileged Cav residues at the positions 8 and 9 of the biologically active NT(8-13) fragment. We analysed the binding modes of the newly synthesized NT(8-13) analogues within the binding pocket of the human neurotensin receptor types 1 and 2 ($NTSR_{1-2}$) using the SeeSAR modelling platform [2,3]. In addition, we studied the chemical stability of selected NT peptidomimetics as well as NT(8-13), the smallest active fragment of N. Basic methodological concepts behind the estimations and associated visualizations are reported in the present study. The applied virtual screening yielded dual $NTSR_1$ and $NTSR_2$ activities of selected peptidomimetics**,** inhibiting in the similar nanomolar range as predicted for NT(8-13). Moreover, the investigated NT(8-13) Cav-based peptidomimetics have been investigated to be chemical and thermodynamically stable under normal conditions for a long-time period.

The investigated *first-in-class* generation of NT(8-13) analogues offer the possibility of broad structural diversities for further virtual and biological screenings on other relevant central nervous system (CNS) targets.

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Keywords*:* Neurotensin mimetics, Parkinson's disease, neurotensin receptors.

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APPLICATION OF MOLECULAR DOCKING PROGRAMS AND VIRTUAL SCREENING FOR DRUG DESIGN

Fatima Sapundzhi[1], Tatyana Dzimbova[1,2]

[1]South-West University "NeofitRilski", Bulgaria, 2700 Blagoevgrad
[2]Institute of Molecular Biology, Bulgarian Academy of Sciences, Bulgaria, 1113 Sofia

In recent years, molecular docking programs played critical role in the design of new drugs. They are widely employed as a fast technique in computer aided drug design. The increasing availability of structural data and high-performance computers expanded the application of these methods. In the current research, we review some molecular docking programs and their application in drug design. Here we consider molecular docking and virtual screening protocols used to predict the drug-receptor interactions. Docking technology is the basic method of computer-aided drug design and will help us to study the bioactive substances and mechanisms of action for treatment of diseases.

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COMBINED ACTION OF HIS-LEU ANALOGUES ON ANGIOTENSIN CONVERTING ENZYME (ACE) AND ANGIOTENSIN RECEPTOR (AR)

Tatyana Dzimbova, Atanas Chapkanov

SWU "Neofit Rilski", 66 Ivan Mihailov str., Blagoevgrad, 2700

Classically, the renin–angiotensin system (RAS) has been viewed solely as a hormonal circulating system involved in the regulation of blood pressure and salt and fluid homeostasis. According to this view, liver-derived circulating angiotensinogen (Aogen) is acting by renin released from the kidney forming the decapeptide angiotensin (Ang) I. Finally, angiotensin converting enzyme (ACE) present on the luminal surface of vascular endothelium converts Ang I to the biologically active end product Ang II by cleavage of the Phe^{8}–His^{9}-bond. This traditional concept has undergone several and important changes in recent years[1]. It has become clear that various fragments of the peptide can act on the receptor. Therefore, the purpose of the present study is to determine by docking whether His-Leu analogues, which act as ACE inhibitors, will have an effect on AR. The compounds were modeled with Avogadro software, the structure of the receptor was obtained from RCSB (PDB id: 4zud), and docking was performed with GOLD 5.2 software. The visualization of the obtained results was performed with a Molegro molecular view, where the energies of the ligand-receptor complexes were determined. The results of the docking indicate that all tested analogs bind to the receptor in an appropriate manner. Three of them – His-sLeu, His-sNle and His-sNle3, have the potential to act as its antagonists, as the formed ligand-receptor complexes have low enough energies to be stable over a long period of time. The test compounds can have a complex effect on RAS, on the one hand by inhibiting ACE and on the other hand by blocking AR.

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DESIGN AND SYNTHESIS OF NEW TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF CHRONIC MYELOID LEUKEMIA AND OTHER MALIGNANT DISEASES

J. Tsonova, I. Ivanov, E. Naidenova

University of Chemical Technology and Metallurgy, "Organic chemistry" Department

Abl-tyrosine kinase signals carry important information about the shape and size of the various cells and hence of the whole organism. If these signals increase or decrease, the balance is disturbed. Abl is a normal cellular enzyme that can be found in all human cells. Bcr-Abl is an oncoprotein that is encoded by a mutant gene in the Ph(+) chromosome. Hyperactive Bcr-Abl-tyrosine kinase activates enzymes involved in protein synthesis. This leads to an uncontrolled growth of myeloid cells evolving to CML. The modern approach to the treatment of this disease is the use of so-called "tyrosine kinase inhibitors" – molecules that directly inhibit Bcr-Abl-tyrosine kinase. Molecular-dynamic simulations show the existence of an intermediate compound between the Abl active and inactive form, which is characterized by an intramolecular bridge between Glu-286 and Arg-386. The aim of the present study is to design and synthesize peptides containing Lys and His which will allow the formation of H-bonds between a His-residue included in the synthesized peptides and Glu-286, as well as biological screening by measuring the proliferation of leukemic cell lines (K-562 and AR-230) in the presence of the resulting peptides.

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Author for correspondence: *Ivan Todorov Ivanov, ivanov_111@abv.bg*

SYNTHESIS AND CHARACTERIZATION OF NEW ANALOGUES OF OCTREOTIDE AND VAPREOTIDE CONTAINING CITRULLINE

Dessislava Borisova, Emilia Naydenova, Boryana Borisova, Dancho Danalev

University of Chemical Technology and Metallurgy, 8 Kliment Ohridski blvd., 1756 Sofia, Bulgaria

One of the main reasons for mortality worldwide, with more than 10 million new cases each year is different types of cancers. In Europe after cardiovascular diseases, malignancies are the second most important illness with about 3 million new cases and 1.7 million deaths annually. Octreotide and vapreotide are synthetic somatostatin (SST) analogues. They have been developed for the treatment of different tumors and flushing episodes associated with carcinoid syndrome as well as diarrhea in patients with vasoactive intestinal peptide-secreting tumors [1]. However, their clinical efficacy is limited due to poor bioavailability and a short half-life in human plasma. This is a result of their fast hydrolysis and metabolism under the action of proteinase in human blood circulation. Many studies have been performed on SST analogues in order to obtain more stable and effective compounds and to establish useful structure-activity relationships [2-4]. It has been reveal that the replacement of the C-terminal carboxyl group with an amide group often leads to molecules with higher biological activity. Herein, we report synthesis and characterization of new C-terminal amide analogues of cyclic SST analogues of octreotide and vapreotide by replacement of natural amino acid Lys with unnatural one citrulline in order to improve the pharmacokinetic profile of the newly synthesized molecules. The aimed peptides were synthesized using SPPS, Fmoc/t-OBu strategy. The intramolecular cyclic disulfide bridge was formed directly on solid phase carrier using specific thallium (III) trifluoroacetate reagent.

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SYNTHESIS AND CHARACTERIZATION OF NEW N-MODIFIED HYBRID HEMORPHIN ANALOGUES

Petar Todorov,* Stela Georgieva, Petia Peneva

University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria,
e-mail: pepi_37@abv.bg

Hemorphins are naturally occurring, endogenous opioid peptides of with affinity for opioid receptors and morphinomimetic properties. They were discovered in the 1980s and include short-chain peptides containing 4 to 10 amino acid residues derived from the β-globin chain of hemoglobin. The hemorphin peptides are increasingly being used in the treatment of various diseases such as hypertension, epilepsy, diabetes, chronic pain, cancer, etc. In order to block the enzymes involving conversion of angiotensin peptides is required searching of non-toxic substances with more efficient anti-inflammatory, anticonvulsant and analgesic activity. That's why, some novel hydantoin-conjugated hemorphin analogues designed were synthesized by solid phase peptide synthesis (SPPS)-Fmoc (9-fluorenylmethoxy-carbonyl) chemistry and physicochemical characterized. The crude neuropeptides were purified on an RP-HPLC and characterized by ESI-MS and FTIR. Structure-activity study will be discussed after investigations of the neurobiological activity of newly synthesized N-modified hybrid analogues.

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SYNTHESIS AND CHARACTERIZATION OF NEW ANTIMICROBIAL FLUORESCENT PEPTIDES FOR ANTIVIRUS PROTECTION OF TEXTILE MATERIALS

Petar Todorov,[a*] Desislava Staneva,[a] Stela Georgieva,[a] Petia Peneva,[a] Ivo Grabchev[b]

[a]University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria,
e-mail: pepi_37@abv.bg
[b]Sofia University "St. Kl. Ohridski", Faculty of Medicine

Herein we report on synthesis and characterization of new antimicrobial peptides, containing fluorophore moiety. The antiviral and virucidal activity have been also discussed. The newly synthesized compounds have potential for antivirus protection of textile materials against human respiratory syncytial virus (HRSV-S2). Peptides with antiviral activity against influenza present the new generation of antiviral drugs with broad-spectrum activity. The great advantage of peptides against viruses and other pathogens consists in the reduced possibility of developing resistance during the treatment. Different methods of textile materials functionalization exist in order to achieve biologically-active effects. The development of a new method has to fulfill the requirements for a safety to human health, and environment. Therefore, the increasing tendency of research is seen where the functionalization is performed by the use of non-toxic, biodegradable, and environmentally-friendly reagents. That's why the aim of this study was to synthesize new fluorescent peptides as potential antivirus protection of textile materials. In order to elucidate the influence of fluorophore moiety, we introduced it to the N-side of hemorphin biopeptides. Herein, the solid-phase synthesis and characterization of new peptides for textile dyeing has been presented. The crude antimicrobial peptides were purified on an RP-HPLC and the molecular weights were determined, using ESI-MS, and also determining of the specific angles of optical rotation. By using combined experimental approaches the structural-textile application has been investigated by UV-Vis and fluorimetric methods.

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METAL IONS INTERACTION WITH AMYLOID-BETA MONOMER AND FIBRIL, A THEORETICAL AND EXPERIMENTAL APPROACH: IMPLICATIONS FOR ALZHEIMER'S DISEASE PROGRESSION

Cosmin Stefan Mocanu[1], Vasile Robert Gradinaru[1], Gabi Drochioiu[1]

[1]*Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi, 11 Carol I Avenue, 70605, Iasi, Romania*

The process of amyloid-beta (Aβ) peptide aggregation, promoted by different forms such as a monomer or fibril, is a known contributor in Alzheimer's disease (AD) pathogenesis. Moreover, the influence of metal ions through amino acid interactions plays a relevant role in the progression of AD.

Furthermore, although the influence hyperpyrexia and consequently, increased intracranial pressure over Alzheimer pathophysiology from a theoretical perspective has not been widely studied, except our study. Therefore, significant modifications have been identified regarding amyloid-beta peptides structures under such symptoms, as well as under the implication of copper ion. Our findings highlight novel variants and pathways affecting cognitive decline, by involving Aβ peptides aggregation in different forms using external factors, such as high pressure of metal ions. Hence, the data obtained outline an *in silico* framework for the generation and analysis of hyperpyrexia influence in neurodegenerative processes that will be applied to future investigation and to specific domains of cognitive deficiencies in order to confirm and expand these findings, such as *in vivo* and i*n vitro* experiments.

In addition, we investigated using an experimental approach, the influence of aluminum ions with Aβ peptide fragments and their analogs. Therefore, our data suggest that the Al strongly induces beta-sheet structures and consequently, can influence AD pathology.

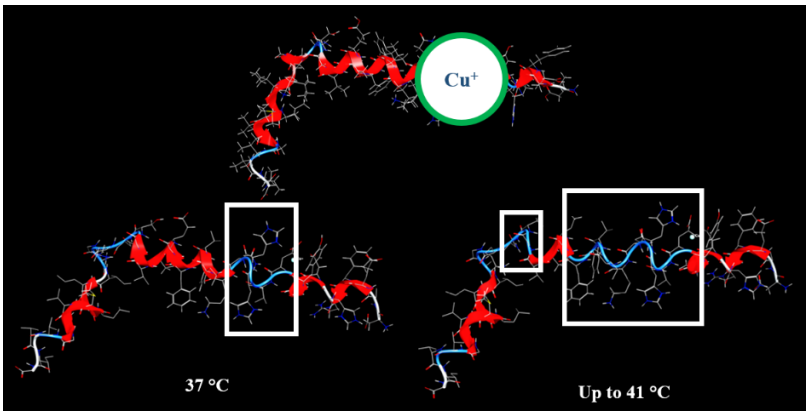

Figure 1. The $A\beta_{(1-42)}$-Cu^{+} complex conformation resulted following the application of molecular dynamics under the conditions imposed (temperature and pressure), at 37 °C (11 mmHg) and 41 °C (20 mmHg).

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THERAPEUTIC EFFECTS OF GLUTATHIONE BIOAVAILABILITY MODULATION IN THE LIGHT OF FERROPTOSIS

T. Georgiev[1], P. Hadzhibozheva[1], Y. Karamalakova[2], K. Georgieva[2], F. Perinkadakatt[3], J. Ananiev[4]

[1]Deptartment of Physiology, Pathophysiology and Pharmacology, Faculty of Medicine, Trakia University – 11 Armeiska Str., Stara Zagora 6000, Bulgaria
[2]Department of Chemistry and Biochemistry, Faculty of Medicine, Trakia University
[3]Medical Student, Faculty of Medicine, Trakia University
[4]Department of Pathology, Faculty of Medicine, Trakia University

Ferroptosis is a recently recognized iron-dependent form of cell death triggered by different agents that inhibit glutathione biosynthesis or the glutathione-dependent antioxidant enzyme glutathione peroxidase 4 (GPX4). As a result, the antioxidant status of the cell is strongly compromised, leading to the activation of certain signaling pathways that cause accumulation of lipid reactive oxygen species and damaging of the membrane structures. Ferroptosis has been reported to be implicated in various disorders, including cancer, neurodegenerative diseases, liver fibrosis, ischemia/reperfusion injuries, and kidney failure. Thus, the manipulation of the process might be beneficial in achieving therapeutic effects: the activation of ferroptosis with specific inducers could result in a destruction of certain tumor cells, while the inhibition with specific ferrostatines may be useful to protect the organs from the damages mentioned above. The present research addresses issues concerning the relationship between ferroptosis and other forms of cell death, and whether activation or inhibition of ferroptosis can be exploited to achieve desirable therapeutic effects.

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PARTICIPATION OF THE OPIOIDERGIC AND NOCICEPTINERGIC SYSTEMS IN THE EFFECTS OF NOCICEPTIN AND NEW ANALOGUES AFTER ACUTE AND CHRONIC IMMOBILIZATION STRESS

Ivelina Himcheva[1], Emilia Naydenova[2], Galiya Stavreva[3], Adriana Bocheva[1]

[1]Medical University of Pleven, Faculty of Medicine, Department of Physiology and Pathophysiology, 1 Sv. Kliment Ohridski Str. 5800 Pleven, Bulgaria
[2]University of Chemical Technology and Metallurgy, Department of Organic Chemistry 8 Kliment Ohridski Blvd.,1756 Sofia, Bulgaria
[3]Medical University of Pleven, Faculty of Pharmacy, Department of Pharmacology and Toxicology, 1 Sv. Kliment Ohridski Str. 5800 Pleven, Bulgaria

Introduction:

Stress is known to exert an influence on neuroendocrine, autonomic, hormonal, and immune functioning (1). Various stress models have been reported to induce analgesia (2-5). This is a phenomenon, referred to as stress-induced analgesia (SIA). Nociceptin and analogues are neuropeptides, neuromodulators, which are able to inhibit the expression of some forms of SIA. Nociceptin/Orphanin FQ(N/OFQ) is a heptadecapeptide which has been found to play a direct role on pain perception. Nociceptin-NOP system modulates several biological functions, stress and anxiety, learning and memory, locomotor activity, food intake (6, 7). The aim of this study was to investigate the effects of nociceptin and novel analogues on nociception after acute and chronic immobilization stress and the involvement of the opioid- and nociceptinergic systems in these effects.

Materials and Methods:

The protected amino acids and Fmoc-Rink Amide MBHA Resin were purchased from Iris Biotech (Germany). All other reagents and solvents were analytical or HPLC grade and were bought from Merck (Germany). The LC/MC spectra were recorded on a LTQ XL Orbitrap Discovery instrument, Thermo Corporation, USA. The optical rotation was measured by automatic standard polarimeter Polamat A, Carl Zeis, Jena.The peptide was obtained as a filtrate in TFA and precipitated with cold dry ether. The compounds were checked by electrospray ionization masspectrometry and the optical rotation was measured in water.

The experiments were carried out on male Wistar rats (180-200g) kept under normal conditions at ambient room temperature (22±2°C), maintained under a 12h/12h light/dark regime, and supplied with standard chow and water *ad libitum*.

Nociception was measured with paw-pressure (PP) and hot-plate (HP) tests. For immobilization stress the animals were placed in a tube; for acute - 1 hours stress procedure and for chronic immobilization stress - 3 hours for 4 days. Nociceptin analogues were synthesized in the laboratory of Prof. Ph.D. E. Naydenova in the University of Chemical Technology and Metallurgy – Sofia. All novel analogues of N/OFQ were injected at a dose of 10 μg/ kg; naloxone (Nal,1 mg/kg), JTC-801 (NOP receptor antagonist, 0,5 mg /kg). Antinociceptive effects were statistically accessed by ANOVA.

Results and Discussion:

Acute immobilization stress increased the pain threshold and hot-plate latency. Naloxone induced hyperalgesia, more pronounced for mechanical pain.

Our results showed that naloxone applied after acute immobilization stress decreased pain threshold and hot-plate latency of N/OFQ(1-13)NH_2, [Orn^9]N/OFQ(1-13)NH_2 and [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2, while for [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 the decrease was statistically significantly more pronounced compared to the [Orn^9]N/OFQ(1-13)NH_2 and acute immobilization stress.

Co-administration of nociceptin and analogues with naloxone decreased statistically significant pain threshold, which is more pronounced than thermal pain.

JTC-801 decreased the pain threshold and hot-plate latency after acute immobilization stress. Nociceptin and analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 administered with JTC-801 after acute immobilization stress decreased the pain threshold and hot-plate latency statistically significantly compared to animals after immobilization stress and JTC-801.

Our results showed that [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 decreased the pain threshold and hot-plate latency statistically significantly more pronounced compared to the [Orn^9] N/OFQ(1-13)NH_2 and acute immobilization stress.

Chronic immobilization stress did not evoke hypoalgesia, but attenuated the hyperalgesic effect of naloxone for mechanical and thermal stimulation, and of naloxone and JTC-801. Simultaneously application of naloxone and JTC-801 reduced the mechanical threshold and thermal pain.

The results suggest that acute and chronic immobilization stress induced hypoalgesia is mediated by opioid receptors and nociceptin neurotransmission; anti-mechanical pain effect is stronger than anti-thermal; the cumulative effect of opioid receptors and nociceptin receptors is observed in the acute stress and in the mechanical modality of the chronic stress.

The cross-talk between nociceptin and opioid systems, supported by anatomical, biochemical and molecular data is revealed (8). The effects of the opioid and nociceptin systems are more pronounced in acute immobilization stress, while the nociceptin mechanisms predominate probably after chronic stress (8).

Conclusions:

For the first time, original results were obtained for the relationships between N/OFQ analogues and the opioid - and nociceptin neurotransmitter systems after acute and chronic immobilization stress. The data suggests that analgesic effects of N/OFQ analogues are influenced by non-selective inhibitor of opioid receptors and inhibitor of (ORL1 or NOP) receptor after acute and chronic immobilization stress.

Our study demonstrated that substitution of Orn at position 9 and 13 in molecule of nociceptine decreased significantly the pain threshold of newly synthesized analogues after acute and chronic immobilization stress.

The newly synthesized analogues of N/OFQ(1-13)NH_2, in which the Lys at the 9th and 13th positions substituted with L-ornithine suppresses the pain threshold more strongly than that of [Orn^9]N/OFQ(1-13)NH_2 after acute and chronic immobilization stress.

In conclusion, the effects of the opioid and nociceptin systems are more pronounced in acute immobilization stress, while the nociceptin mechanisms predominate probably after chronic stress.

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THE ENDOGENOUS CANNABINOID SYSTEM AND NITRIC OXIDE INTERACT IN MODULATION OF COLD STRESS-INDUCED ANALGESIA

Hristina Nocheva, Georgi Bogdanov, Roman Tashev, Rumen Nikolov

Medical University of Sofia, Zdrave Str. 2, Sofia, Bulgaria

Stress-induced analgesia (SIA) represents an indirect indicator for modulation of the stress reaction. Understanding the underlying mechanisms of such reaction could give helpful clues for decreasing the sometime deleterious effect of the stress impact. Both the endogenous cannabinoid system (ECS) and nitric oxide (NO) are known to take part in the development of the stress-reactions.

The aim of the present study was to estimate the effect of the ECS and the NO-ergic system interaction on SIA.

The CB1-receptor agonist (anandamide, AEA) and the NO precursor L-arginine (L-arg) were intraperitoneally administrated before or after one hour of cold stress (1h CS). CB1 antagonist AM251 and the NO-synthase inhibitor L-NAME were additionally applied in order to further elucidate the interaction between the two systems. Pain thresholds were estimated through Paw pressure (PP) test.

AEA administration before 1h CS increased cold-SIA during the first 20 min of the experiment. Administration of L-arg decreased paw-pressure thresholds. AM251 prevented cold-SIA potentiation, while L-NAME led to a prominent long-lasting increase of paw-pressure thresholds. Administration of NO-donor SIN-1 after L-NAME decreased paw-pressure thresholds resembling the effect of L-arg.

AEA administration after 1h CS decreased PP-thresholds of experimental animals compared to 1h CS. Administration of L-arg along with AEA led to a short-lasting increase of cold-SIA on the 10^{th} min of the experiment. Administration of AM251 or L-NAME prevented the abovementioned increase in cold-SIA and after the 20^{th} min PP-thresholds showed a tendency toward hyperalgesia compared to the controls.

The results obtained point at a different interactions between ECS and NO in mediation (when applied before 1h CS) and modulation (when applied after 1h CS) of stress-induced analgesia.

Key words: cold stress-induced analgesia, endocannabinoids, L-arginine, nitric oxide, Paw-pressure test

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LATERALIZED ACTIVE AVOIDANCE LEARNING AND MEMORY TO ANGII AND LOSARTAN MICROINJECTED INTO AMYGDALA IN RATS DEPRESSION MODEL

Roman Tashev[1], Hristina Nocheva[1], Margarita Ivanova[2]

[1]Department of Pathophysiology, Medical University of Sofia, Zdrave Str., 2., Sofia, Bulgaria
[2]Department of Physiology and Pathophysiology, Medical University of Varna, M. Drinov Str. 55, Varna, Bulgaria

Depression is a widespread socially disease. Studies aimed at detecting the pathogenesis of depression have lasted for decades, but specific pathogenetic mechanisms remain unclear. Olfactory bulbectomy (OBX) in rats provides a well-validated animal model of depression and Alzheimer's disease.

The main biologically active peptide of RAS-angiotensin II (Ang II) binds to specific AT1 and AT2 receptors that have uneven distribution in CNS.

There is strong evidence that two hemispheres are differentially involved in emotional memory and that amygdala has a key role. The aim is to study the involvement of Ang II and AT1 receptors in learning and memory after unilateral infusion into CeA (central nucleus of the amygdala) in rats with a model of depression. The effects of Ang II (0.5 μg) and losartan (specific AT1 antagonist - 100 μg) infused into CeA on the avoidance performance in OBX rats using active avoidance (shuttle box) test were investigated. The stereotaxic technique was used for bilateral implantation of guide cannulas into CeA of the OBX rats. After a 14-day recovery period, rats were microinjected with Ang II or losartan unilateral on the background of developed depression-like behavior.

For the first time it was found that Ang II infused into left CeA on the background of depression-like behavior impaired learning and memory, while losartan infused into left CeA significantly improved these processes and that reverses memory deficits induced by bulbectomy unlike Ang II.

The data suggest an involvement of amygdalar Ang II and AT1 receptors in learning and memory of rats and a differential distribution of AT1 receptors in the left and right central nucleus of the amygdala in rats with model of depression.

Keywords: angiotensin II, losartan, learning, memory, asymmetry, amygdala, depression, olfactory bulbectomy

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