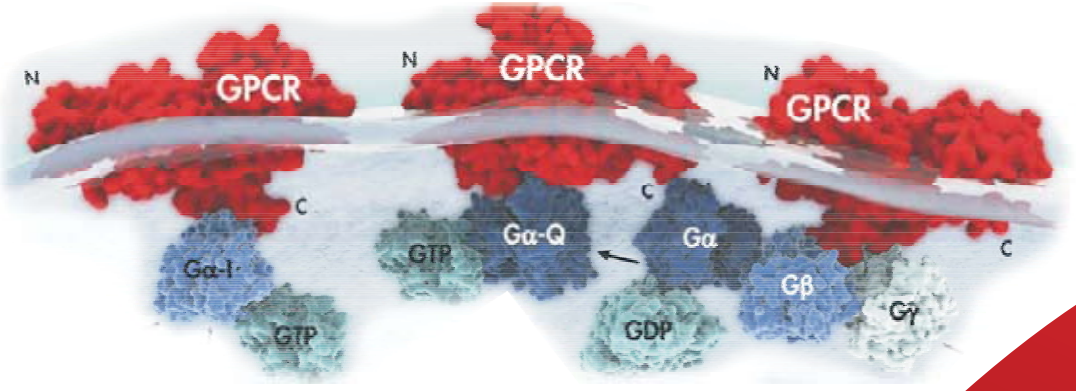
8th Biannual Meeting of the Hellenic (Greek) Society for Basic & Clinical Pharmacology



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IUPHAR IMMUNOPHARMACOLOGY SECTION

Athens 23-24 May 2014

ΚΑΘΕ ΣΤΙΓΜΗ ΠΟΥ ΧΑΙΡΕΣΤΕ ΤΗ ΖΩΗ
ΕΙΜΑΣΤΕ ΕΚΕΙ ΚΙ ΑΣ ΜΗΝ ΤΟ ΞΕΡΕΤΕ

Στη ζωή υπάρχουν πράγματα που δεν τα βλέπουμε, αλλά τα νιώθουμε. Όπως τη φροντίδα της SANOFI. Γιατί καθημερινά βρίσκεται στο πλευρό σας με ΚΑΙΝΟΤΟΜΑ ΠΡΩΤΟΤΥΠΑ και ΓΕΝΟΣΗΜΑ ΦΑΡΜΑΚΑ που βοηθούν να βελτιώσετε την υγεία και την ποιότητα της ζωής σας. Και με την εμπιστοσύνη σας μπορεί και συνεχίζει αυτό που ξεκίνησε πριν από 2 αιώνες. Να ερευνά, να καινοτομεί και να προσφέρει σε εκατομμύρια ανθρώπους σε όλο τον κόσμο ό,τι πολυτιμότερο υπάρχει στη ζωή. Υγεία!

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Γιατί η ουσία είναι στην υποχρεωση

Final Programme

Friday May 23

8.30-9.00	Registration
	Young Investigators Forum Chairs: Dr. E. Papadimitriou, Dr. C. Tamvakopoulos
9.00-9.15	Interaction of pleiotrophin with vascular endothelial growth factor receptor 2. M. Lamprou, E. Papadimitriou
9.15-9.30	The effect of the olive constituent, oleuropein, on lipid homeostasis: role of PPARα. F. Malliou, I. Andreadou, K. Kypreos, M. Marselos, E. Iliodromitis, L. Skaltsounis, M. Konstandi
9.30-9.45	Endogenous cannabinoids and the inhibitors of their metabolic enzymes protect the retina from AMPA-induced excitotoxicity *in vivo*. D. Kokona, A. Makriyiannis, A. Zimmer, K. Thermos
9.45-10.00	Differential induction of ALDH1A7 in RR and rr rats is associated with the promoter structure, but not with nuclear receptor functionality. K. Touloupi, P. Honkakoski, M. Marselos, P. Pappas
10.00-10.15	Association of ABCB1 gene polymorphisms with dyslipidemia. A.Panderi, D. Agapakis, C. Savopoulos, D. Kouvelas, AI. Hatzitolios, A. Goulas
10.15-10.45	*Coffee Break*
	IUPHAR Immunopharmacology Section (sponsored and hosted by the Greek Pharmacological Society) Chairs: Dr. K.Tyligada, Prof. F. Levi-Schaffer
10.45-10.50	Katerina Tiligada, PhD (University of Athens, Greece) The newly founded Immunopharmacology Section of IUPHAR
10.50-11.20	Francesca Levi-Schaffer, PhD (The Hebrew University of Jerusalem, Israel, Chair of IUPHAR Immunopharmacology Section): In vitro & in vivo models in immunopharmacology: Open-minded for surprises!
11.20-11.50	Madeleine Ennis, PhD (The Queens University of Belfast, UK): Mast cells – important not only in allergic diseases but also other diseases!
11.50-12.20	Anastassios Germenis, MD, PhD (University of Thessaly, Greece): Design of agonistc altered peptides for immunothepary: the dawn of Quantum Immunology

12.20-13.50	*Lunch*/Poster viewing
	Academia-Industry partnerships and interactions: challenges and opportunities Chairs: Prof. Z. Papadopoulou-Daifoti, Prof. M. Maragoudakis
13.50-14.20	Dimitrios Tzalis, PhD (Taros Chemicals GmbH): Funding opportunities for Pharmacologists through the European Lead Factory (an Innovative Medicines Initiative)
14.20-14.50	Dimitrios Demos, MBA (President, Panhellenic Union of Pharmaceutical Industry) Funding opportunities and interactions with industry
14.50-16.20	Round table discussion organized by the Hellenic Association of Pharmaceutical Companies (SFEE) Barbara Baroutsou, MD, PhD (Head of Medical Directors Committee): Biomedical research and pharmaceutical industry Aggelos Tsakanikas, PhD (University of Athens, Greece) Research and Innovation as a strategic plan for Greece Kostas Athanasakis, PhD (National School of Public Health) Clinical Research in Greece Vicky Soulandrou (European Public Law Organization) Removing the obstacles to attract more clinical trials in Greece
16.20-17.00	*Coffee Break*
	Publishing and peer-review Chair Prof. C. Flordellis
17.00-17.30	Jaap Harten, Ph.D. (Elsevier) Research misconduct and publishing ethics
	Hot topics Chairs: Prof. N. Sitaras, Prof. K.Thermos
17.30-18.00	Elias Lolis, PhD (Yale University, USA): Macrophage migration inhibitory factor, associated diseases, and its inhibition
18.00-18.30	Antonis Tsarbopoulos, PhD (University of Athens, Greece): Therapeutic Proteins: The Hunt for Post Translational Modifications
18.30-19.00	Vangelis Manolopoulos, PhD (University of Thrace, Greece): Genotype-guided dosing of anticoagulants
	Keynote Lecture Chair: Prof. A. Papapetropoulos
19.00-20.00	Arthur Christopoulos, PhD (Monash University, Australia): GPCR Allostery and Bias in Drug Discovery
20.00-	Welcome reception

Final Programme

Saturday May 24

Pharmacology education
(Session organized by the British Pharmacological Society)
Chairs: Prof. R. Flower, Dr. I. McFadzean

9.00-9.30 Ian McFadzean, PhD (King's College London, UK):
How the BPS works to promote education in pharmacology within the UK

9.30-10.00 Jess Strangward (British Pharmacological Society): Prescribing Safety Assessment: Raising the profile of Clinical Pharmacology in the UK

10.00-10.30 Stephen Alexander, PhD (University of Nottingham, UK):
The IUPHAR/BPS guide to pharmacology

10.30-11.00 Rod Flower, PhD (William Harvey Research Institute, UK)
"Pharmacology 2.0"

11.00-11.30 *Coffee break*

Translating basic pharmacology to drug development
Chairs: Prof. A. Gravanis, Prof. N. Sakelaridis

11.30-12.00 Anastassis Perrakis, PhD (The Netherlands Cancer Institute, Amsterdam)
Autotaxin: from function and structure towards drugs, and back again

12.00-12.30 Dimitris Skokos, PhD (Regeneron Pharmaceuticals, Inc., NY, USA)
Targeting the Delta-4 Notch ligand

12.30-13.00 Andreas Papapetropoulos, PhD (University of Athens, Greece):
CBS as a potential target in colon cancer

13.00-14.00 *Lunch*/Poster viewing

Cardiovascular pharmacology
Chairs: Prof. D. Kouvelas, Dr. I. Andreadou

14.00-14.30 Eddie Weitzberg, PhD (Karolinska Institute, Sweden): Nitrate and nitrite: the journey from degradation products to promising therapeutics

14.30-15.00 Kyriakos Kypreos, PhD (University of Patras, Greece):
Revisiting the role of HDL in cardiovascular disease

15.00-15.30 Fabio Di Lisa, PhD (University of Padova, Italy):
The role of mitochondria in heart disease

15.30-16.00 Stavros Konstantinides, MD, PhD (University of Thrace, Greece):
New oral anticoagulants: mechanisms of action, pharmacological properties and clinical data

16.00-16.30 *Coffee Break*

	Neuropharmacology: Towards translational neuropsychopharmacology: Focus on neural plasticity theory of Nervous System Disorders Chairs: Prof. P. Papaioannidou, Dr. K. Antoniou
16.30-17.00	Catherine Belzung, PhD (UFR Sciences et Techniques, INSERM 930 and University Francois-Rabelais, Tours, France): Neurogenesis, Stress and Depression
17.00-17.30	Christina Dalla, PhD (University of Athens, Greece): Neuroplasticity modulation: effects of stress, gender and antidepressants
17.30-18.00	Ioannis Charalampopoulos, PhD (University of Crete, Greece): Novel synthetic microneurotrophins: potential therapeutic applications in nervous system
18.00-18.30	Awards and Prizes-EPHAR Poster Award
20.00-	Official dinner

General Information

Congress Venue:	The Biomedical Research Foundation of the Academy of Athens Soranou Efesiou 4, 115 27 Athens Greece
Phone:	00302106597000, email: info@bioacademy.gr, www.bioacademy.gr
Organising Secretariat:	IMAGINE CONGRESS & TRAVEL 9, Kolokotroni street 105 62 Athens Greece/ Tel.: 2103252520, Fax. : 2103252530 E mail: imaginecongress@otenet.gr, www.imaginecongress.gr
Official Language:	The official language of the Congress is English.
Badges:	Badges will be issued upon checking your registration at the Secretariat Desk.
Registration Fees:	Regular 30,00€ Msc/PhD students 20,00€ Undergraduate students: free
Certificate of Attendance:	Certificates will be sent by e mail to the participants after the Congress.
Coffee and Refreshments:	Coffee and refrushments will be available at the exhibition area during official breaks.

Notes

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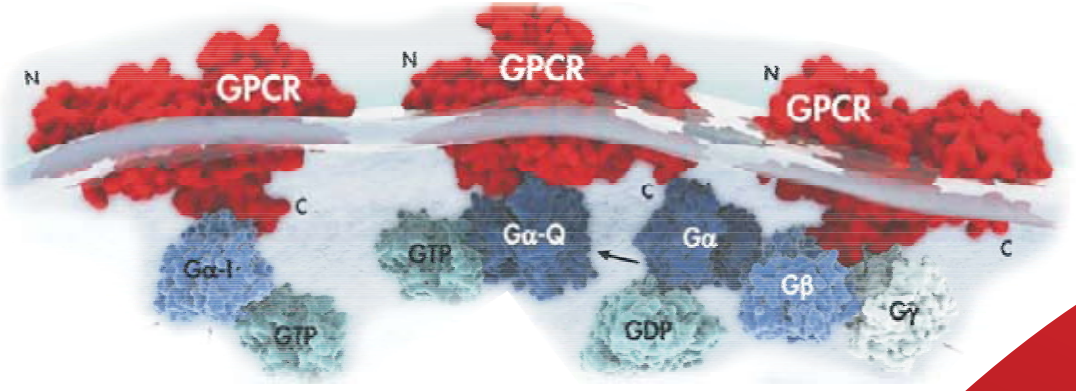
Πριν τη συνταγογράφηση συμβουλευθείτε την Περίληψη των Χαρακτηριστικών του Προϊόντος που διατίθεται στο εκθετήριο

8th Biannual Meeting of the Hellenic (Greek) Society for Basic & Clinical Pharmacology



Keynote speaker:
Professor Arthur Christopoulos,
B.Pharm., Ph.D., Drug Discovery,
Monash University, Australia

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IUPHAR IMMUNOPHARMACOLOGY SECTION

ABSTRACT BOOK

Athens 23-24 May 2014

Η αποκωδικοποίηση του DNA ανοίγει κάθε μέρα νέους δρόμους θεραπείας. Όσο η επιστήμη θα παράγει διαρκώς νέα γνώση, τα όρια της υγείας θα διευρύνονται.
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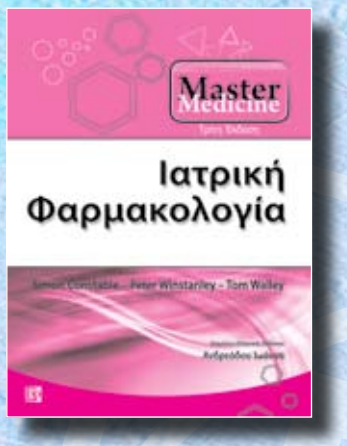

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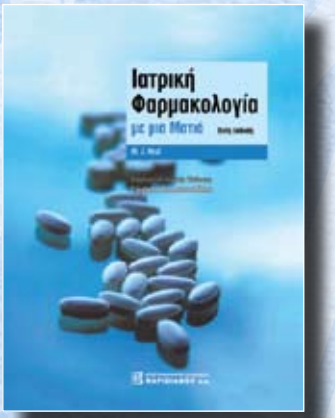

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PP-01

A SINGLE *IN VIVO* EXPOSURE TO FENTANYL INDUCES LONG-LASTING REDUCTION OF GABAERGIC INHIBITION IN THE CA1 AREA OF THE VENTRAL HIPPOCAMPUS.

E. Kouvaras, N. Pipidou, A. Kantikou and E.K. Asprodini.
Laboratory of Pharmacology, Medical School, University of Thessaly, Larissa, Greece.

BACKGROUND & OBJECTIVES

The hippocampal formation is differentiated along its longitudinal axis with the ventral pole being more susceptible to epileptiform discharges. We have shown previously that *in vivo* administration of fentanyl reduces GABAergic inhibition and increases excitability in the CA1 area of the dorsal hippocampus. It was the purpose of the present study to examine the effect of *in vivo* fentanyl treatment on the inhibitory transmission and excitability of CA1 pyramidal neurons of the ventral hippocampus.

METHODS

Male Wistar rats were treated either with saline or fentanyl (4x80 μg/kg s.c. every 15min). One day after treatment, standard intracellular recordings were obtained from 500μm-thick transverse ventral hippocampal slices of saline- and fentanyl-treated animals. Synaptic responses were evoked by stimulation of Schaffer collaterals. Comparisons between treatment groups were made with the Student's *t* test.

RESULTS

Fentanyl treatment did not alter basic passive and active membrane characteristics (resting membrane potential (RMP), input resistance, postsynaptic firing patterns) of pyramidal neurons. The peak amplitude of postsynaptic fast (f-) and slow (s-) inhibitory potentials (IPSPs), measured at 43.6±2.9 ms and 163.2±12.7 ms, respectively, from the onset of the synaptic response, was smaller in neurons from fentanyl- (f-IPSP: 2.1±0.9mV; s-IPSP: 1.1±0.3, n=4) compared to saline-treated animals (f-IPSP: 7.3±1.8mV; s-IPSP: 5.8 ±0.5mV, n=4; $p<0.05$) when measured at RMP (saline-treated: -65.0±1.0mV, fentanyl-treated: -65.7±1.8mV; $p=0.38$).

CONCLUSIONS

In accordance to our previous findings from the dorsal pole of the hippocampus, preliminary data from the ventral pole points to the reduction of GABA-mediated inhibition 24 hours following *in vivo* exposure to fentanyl.

Acknowledgements: This work was supported by the Ministry of Education (Herakleitos II).

PP-02

DEVELOPMENT OF LC-MS/MS AND LC-UV BASED ANALYTICAL METHODS FOR DRUG QUALITY CONTROL ASSSESMENT

P. Agrafiotou, C. Tamvakopoulos
Division of Pharmacology & Pharmacotechnology, Biomedical Research Foundation, Academy of Athens

BACKGROUND & OBJECTIVES

The drug quality control process is not only important for getting a new drug approved but it is also an important process for ensuring that drugs that have reached the market can be periodically monitored in order to ensure their stability. For that reason the development of validated analytical methods is critical and includes procedures, which demonstrate that a particular method used for determination of analytes in a drug formulation (tablet), is reliable and reproducible for the intended use. The fundamental parameters for this purpose include determination of accuracy, precision, selectivity, sensitivity, reproducibility, LLOQ and stability.

METHODS

High performance liquid chromatography (HPLC) connected to a UV/Vis detector was used in order to develop and validate methods for the identification and quantification of three known drugs: acetaminophen, omeprazole and meloxicam in pharmaceutical tablets. Furthermore, LC system connected to a hybrid triple-quadruple/linear ion trap mass spectrometer was used for additional evaluation of the drugs examined in terms of quality and purity.

RESULTS

Pharmaceutical tablets containing 15 mg meloxicam (from 5 different manufacturers), 20 mg omeprazole (5 manufacturers) and 500 mg acetaminophen (4 manufacturers) were dissolved in the appropriate solvent, filtered and then examined in terms of the quantity of the drastic substance and the purity of the drug using the developed validated methods.

CONCLUSIONS

HPLC methods were developed and validated for the determination of meloxicam, acetaminophen and omeprazole and were applied successfully to commercially available tablets. The described methods can be readily used for routine quality control of tablets.

PP-03

DUAL TARGETING OF TUMOUR ANGIOGENESIS AND GNRH-R EXPRESSING PROSTATE CANCER CELLS, USING NOVEL SUNITINIB-GNRH CONJUGATES.

O. Argyros[1], T. Karampelas[1], X. Asvos[2], D. Fokas[3] and C. Tamvakopoulos[1]
[1]*Division of Pharmacology-Pharmacotechnology, Biomedical Research Foundation Academy of Athens (BRFAA),*
[2]*Laboratory of Medicinal Chemistry, Department of Chemistry, University of Ioannina, Greece*
[3]*Laboratory of Medicinal Chemistry, Department of Materials Science and Engineering, University of Ioannina, Greece*

BACKGROUND-OBJECTIVES

The Gonadotropin Releasing Hormone Receptor (GnRH-R) is being pursued as a target in prostate cancer (CaP) for the selective delivery of GnRH-drug conjugates. Here we present the synthesis and evaluation of novel **S**unitinib **AN**alogues (SANs) that can be conjugated to GnRH in order to specifically target both the GnRH-R1 expressing cells as well as angiogenesis in the tumour microenvironment. Crucially, our analogues can be readily conjugated to multiple targeting peptides in order to treat other types of cancers.

METHODS

Several novel SANs were synthesized to allow for direct conjugation of GnRH to Sunitinib. *In vitro* molecular targeting and cytotoxicity of SANs were examined in HUVEC, NIH/3T3 and androgen-independent CaP cell lines (DU145, PC3) and in transgenic DU145 and PC3 cell lines engineered to stably express GnRH-R1. Initial pharmacokinetic studies were performed in mice and blood samples were analysed by LC-MS/MS.

RESULTS

All SANs proved efficacious in the *in vitro* assays performed, with one specific SAN (named SAN2), displaying strong inhibition of its molecular targets VEGFR2 and PDGFRβ along with high anti-proliferative activity against all cell lines. Pharmacokinetic studies following intraperitoneal delivery revealed that SAN2 shows favorable properties that justify its further development.

CONCLUSIONS

The newly synthesized SAN2 displayed significant efficacy advantages *in vitro* compared to equimolar doses of other SANs and proved equipotent to the original Sunitinib. The development in our group of GnRH-R1 stable CaP cell lines and further conjugation of SAN2 to GnRH will allow us to proceed with proof-of-concept *in vivo* efficacy and targeting experiments.

PP-04

SOLUBLE GUANYL CYCLASE (sGC) ACTIVATION BY NO AND HEME-INDEPENDENT ACTIVATORS: EVIDENCE FOR DIFFERENTIAL REQUIREMENTS OF RESIDUES IN THE β1 REGULATORY DOMAIN

Z. Zhou[1], A. Marazioti[2], S. Topouzis[2], A. Giannis[3], G. A. Spyroulias[2], and A. Papapetropoulos[1,4]
[1]*"G.P.Livanos-M.Simou" Laboratories, 1st Department of Critical Care Medicine and Pulmonary Services, Evangelismos Hospital, School of Medicine, University of Athens, Greece*
[2]*Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Greece*
[3]*Institut für Organische Chemie, Universität Leipzig, Germany*
[4]*Department of Pharmacy, University of Athens, Greece*

BACKGROUND & OBJECTIVES

sGC is a heterodimeric α/β hemoprotein that regulates signalling in response to nitric oxide (NO). We compared the contribution of the β1 regulatory domain to NO and two chemically distinct heme-independent activators, namely HMR-1766 and BAY 58-2667.

METHODS

Using computational approaches, we identified residues in the N-terminus of β1 that might influence the response to HMR-1766 and BAY 58-2667. After mutagenesis, sGC function was analyzed in transient transfection experiments.

RESULTS

While exposure of wt sGC-overexpressing cells to HMR1766 led to higher cGMP levels, BAY 58-2667 was a more potent sGC activator. Mutating His to Phe at β1 105 abolished sGC responsiveness to sodium nitroprusside (SNP), due to heme loss. In contrast, BAY 58-2667 and HMR-1766 activated heme-free H105F sGC. Simultaneous mutation of Y135/R139 to Ala lead to an enzyme that was unresponsive to both NO and sGC activators. D45E, P74H and Y83G mutants exhibited a reduced ability to synthesize cGMP in response to SNP and HMR-1766. The responsiveness L142G to SNP was reduced, while the effect of HMR-1766 remained unaffected. The R116A mutant exhibited unaltered cGMP-synthesizing ability for HMR-116, a mild reduction towards SNP, but increased cGMP generation after BAY 58-2667 exposure. P118A exhibited reduced responses to SNP and HMR-116, but unaltered behaviour towards BAY 58-2267. Exposure of wt enzyme to the heme oxidant ODQ reduced sGC levels; this effect was totally reversed by BAY 58-2667, but only partially by HMR-1766.

CONCLUSIONS

We have identified key residues that are important for the differential responsiveness of sGC to NO/heme-independent sGC activators and NO-releasing drugs.

PP-06

IDENTIFICATION AND DEVELOPMENT OF STRUCTURALLY NOVEL INHIBITORS AGAINST THE H1047R MUTANT OF PI3Kα

E. Kouvari[1], O. Argyros[1], P. Marakos[2], N.Pouli[2], N. Lougiakis[2], S. Christoforidis[3], E. Mikros[2], L. Skaltsounis[4], C. Tamvakopoulos[1]

[1]Division of Pharmacology-Pharmacotechnology, Center of Basic Research, Biomedical Research Foundation, Academy of Athens, Athens, Greece.

[2]Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Athens, Athens, Greece.

[3]Laboratory of Biological Chemistry, School of Medicine & Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology (BR-IMBB/FORTH), University of Ioannina, Ioannina, Greece.

[4]Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece.

BACKGROUND & OBJECTIVES

PIK3CA gene, coding for the catalytic subunit of PI3Kalpha (p110α), is one of the most frequently mutated oncogenes in human tumors. Although several inhibitors against the wild-type PI3Kα enzyme are currently in clinical trials, none of them is selective for the mutated form, increasing the risk for potential side effects. Here, we present our efforts to develop novel inhibitors, targeted against the most common mutation of p110α (H1047R), using a library available in the University of Athens, containing natural products and novel synthetic compounds.

METHODS

Evaluation of the cytotoxicity of 275 compounds of various chemical scaffolds against human breast cancer cell lines was performed using an *in vitro* cell viability assay (MTT). A modified cell-free assay was employed to assess the selectivity of potential inhibitors against the wild-type or the H1047R mutated enzyme. We also performed initial Pharmacokinetic evaluation of lead compounds using Liquid Chromatography-Tandem Mass Spectrometry techniques.

RESULTS

Pyridopyrazine 1043 showed potency in the MTT assay (IC_{50}~10μM) and the cell-free assay (IC_{50}~15μM), but no selectivity for the H1047R mutation. Pyrazolopyridines 1113 and 1119 also proved to be very potent but not selective PI3Kα inhibitors in all the *in vitro* assays with an IC_{50} at the low micromolar level. 1043 is orally and intraperitoneally bioavailable in mice and showed good blood exposure after a single dose of 5mg/kg, Cmax(2h)=1.7μM.

CONCLUSIONS

Several potent PI3Kα inhibitors were discovered, with favorable pharmacokinetic properties at preliminary doses. These compounds represent lead chemical structures, certainly worthy of further evaluation and optimization for the development of specific H1047R inhibitors.

PP-07

3D VASCULARIZED TUMOUR SPHEROIDS: AN IN VITRO MODEL OF CANCER ANGIOGENESIS

O Tsigkou[1], PK Politis[2] and C Tamvakopoulos[1]
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BACKGROUND & OBJECTIVES

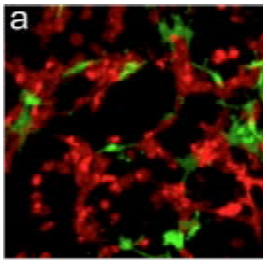
Angiogenesis, the formation of new vessels, is crucial for tumour progression and metastasis. Typical monolayer cell culture (2D) assays have offered tremendous insight in fundamental cancer biology and drug development. However, the microenvironment within which cells reside and interact with the tumour extracellular matrix is more complex. The objective of this study is to develop 3D vascularized tumour spheroids (TμSph) that recapitulate the tumour microenvironment and improve the *in vitro* assessment of cancer therapeutics.

METHODS

For TμSph formation, HUVECs, MSCs and human breast cancer and hepatoma cell line (MDA, MCF-7, HepG2 and Huh-7) transduced with lentiviruses, were mixed in a fibronectin containing collagen gel. TμSph were treated with various concentrations of sorafenib and cilengitide and their effect on cell metabolic activity, angiogenesis, migration and metastasis was assessed *in vitro*.

RESULTS

Microscopy demonstrated the formation of capillary-like network within the TμSph with lumen like structures (Figure 1). TμSph treated with 0-50μM sorafenib and 0-200μM cilengitide demonstrated that higher drug concentrations are necessary for destruction of the vascular network, cell migration and toxicity compared to 2D cell cultures. Gene (real time Reverse Transcription-Polymerase Chain Reaction (rt RT-PCR) and protein (Western Blotting) expression profiles and signalling pathways affected by the drugs, and how these compare to 2D cultured cells will also be presented.



CONCLUSIONS

TμSph pose a more promising in vitro drug-screening model compared to 2D standard cell culture assays.

PP-08

TARGETED MASS SPECTROMETRY-BASED METHODOLOGIES FOR THE DETERMINATION OF LIPID BIOMARKERS ASSOCIATED WITH ALLERGIC AIRWAY INFLAMMATION & ITS RESOLUTION

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BACKGROUND & OBJECTIVES

Ω3 and Ω6 polyunsaturated fatty acid (PUFA)-derived lipid mediators (LM) have a significant contribution in the homeostasis but also in pathological inflammatory conditions of the lung. LM have been implicated in multiple inflammatory mouse models. Such studies attributed specific functions to different families of LM; pro-inflammatory LM are responsible for the mounting of an acute inflammatory response while specialized pro-resolving lipid mediators (SPMs) regulate its timely resolution. The objective is to study the biosynthesis of selected LM in a pre-clinical model of allergic airway inflammation (AAI) and further evaluate their use as potential biomarkers.

METHODS

AAI was induced in C57Bl/6 mice following sensitization with ovalbumin (OVA) and challenges with nebulized OVA. Tissues were collected during the resolution phase. LC-MS/MS methods were developed an applied towards the determination of 13 selected LM with relevance to AAI, in serum and lung tissue.

RESULTS

Highly sensitive LC-MS/MS methods developed were validated for application in mouse serum and lung. Analysis of lung tissue obtained during the resolution phase of the AAI model, revealed increased concentrations of Protectin D1 (PD1) and Protectin DX (PDX) only in mice with inflamed airways. Their direct upstream biosynthetic precursor, 17-HDHA, was also upregulated.

CONCLUSIONS

The present study investigated the kinetics of LM production in a self-resolving mouse model of AAI. SPMs of the Protectins' family showed good correlation with the resolution phase suggesting their potential use as biomarkers of this process. These findings may be of value in clinical conditions were pharmacological intervention will be required.

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* C.T and E.A share authorship.

PP-09

NOVEL GNRH-GEMCITABINE CONJUGATES FOR THE TREATMENT OF ANDROGEN-INDEPENDENT PROSTATE CANCER: TARGETED DRUG DELIVERY COMBINED WITH PHARMACOKINETIC ENHANCEMENTS

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BACKGROUND AND OBJECTIVES

Gemcitabine, a drug with established efficacy against a number of solid tumors, has therapeutic limitations due to its rapid metabolic inactivation. The aim of this study was the development of an innovative strategy to produce a metabolically stable analogue of gemcitabine that could also be selectively delivered to prostate cancer (CaP) cells based on cell surface expression of the Gonadotropin Releasing Hormone-Receptor (GnRH-R).

METHODS

The synthesis and evaluation of conjugated molecules, consisting of gemcitabine linked to a GnRH agonist, is presented along with results in androgen-independent prostate cancer models. NMR and ligand binding assays were employed to verify conservation of microenvironments responsible for binding of novel GnRH-gemcitabine conjugates to the GnRH-R. *In vitro* cytotoxicity, cellular uptake and metabolite formation of the conjugates were examined in CaP cell lines. Evaluation of efficacy of selected candidates with the best pharmacokinetic properties was based on a GnRH-R positive androgen-independent CaP animal model.

CONCLUSIONS

Selected conjugates were efficacious in the *in vitro* assays with one of them, namely GSG, displaying high antiproliferative activity in CaP cell lines along with significant metabolic and pharmacokinetic advantages in comparison to gemcitabine. Finally, treatment of GnRH-R positive xenografted mice with GSG, showed a significant advantage in tumor growth inhibition when compared to gemcitabine.

PP-10

PRESCRIPTION MEDICATIONS AND HERBAL DRUGS INTERACTIONS

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ABSTRACT

BACKGROUND AND OBJECTIVES

Plants have been used for disease treatment since Hippocrates era and in recent years the consumers preference towards herbal preparations presents a remarkable increase. The herbal supplements and alternative treatments play a significant role in patients' choice for health maintenance, better physical status, disease fight and cosmetic purposes. Both prescription medications and medicinal plants exert a pharmacodynamic effect on biological systems and their concurrent administration may cause severe side effects due to interference interactions. Herbs are organisms containing various active ingredients that exhibit changes on the biological substrate. Concurrent use of herbs may mimic, magnify, or oppose the effect of drugs. Potential herb-drug interactions include: bleeding when warfarin is combined with plants contained coumarin derivate like ginkgo (Ginkgo biloba) garlic (Allium sativum and other) or serotonin-reuptake inhibitors with St John's wort (Hypericum perforatum) Moreover St John's wort decreased bioavailability of digoxin, theophylline, cyclosporin, and phenprocoumon; combined antidepressants and Panax ginseng induce mania in depressed patients; anthranoid-containing plants (senna and cascara) and soluble fibres (psyllium) can decrease the absorption of drugs. Health-care practitioners should caution patients against mixing herbs and pharmaceutical drugs.
The aim of this paper was to investigate the interactions between prescription medications and herbal drugs

METHODS

Extensive literature survey on herbs - drugs interactions.

RESULTS

Many reports of herb-drug interactions lack laboratory analysis of suspect preparations. The mechanisms of interactions are induction of cytochrome P450 and isoenzymes CYP3A4, CYP2C9, CYP1A2.

CONCLUSIONS

Health care practitioners should be aware of the herbal properties and discourage patients, who take drugs with narrow therapeutic index (warfarin, digoxin, hypoglemic agents, theophylline, antidepressants) to use herbal products.

PP-11

IN VITRO SLICE PREPARATION – RELATING FUNCTION TO LABELLING

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BACKGROUND & OBJECTIVES

Coupling of neuronal functional characteristics to specific markers and morphological attributes has always been challenging in neuroscience research. Based on our recent findings that immersion fixation of hippocampal slices exhibits satisfactory histological and immunohistochemical staining, we used the *in vitro* slice preparation to study functional GABAergic inhibition in relation to GABA immunolabelling.

METHODS

Hippocampal transverse slices (500μm thick) were prepared from male Wistar rats and maintained in oxygenated ACSF. Sharp-electrode intracellular recordings were obtained from CA1 pyramidal neurons; synaptic responses were evoked by stimulation of Schaffer collaterals. At the end of the electrophysiological recordings (3-8 hours post brain excision), slices were fixed by immersion (4% PFA/0.1M PB) and embedded in paraffin. Consecutive paraffin 4μm-thick slices were stained histologically using Eosin/Hematoxylin or immunohistochemically for GABA using primary polyclonal rabbit antibody against GABA (1:500) and fluoroscein-conjugated secondary goat anti-rabbit IgG (H+L) (1:400).

RESULTS

Neurons exhibited basic electrophysiological passive and active membrane properties (resting membrane potential, input resistance, postsynaptic firing patterns), similar to previously described. Pronounced GABA-mediated synaptic inhibition was associated with the presence of numerous GABA-immunofluorescent neurons in stratum radiatum. Within the same layer, Eosin/Hematoxylin-stained slices demonstrated neurons with normal ultrastructure and clear and evident nuclei and nucleoli.

CONCLUSIONS

Although preliminary, our results suggest that immersion fixation may be used for the histological/immuhistochemical study of tissue which has been previously studied electrophysiologically *in vitro*. The broader significance of this method is that neuronal function can be directly related to diverse markers and mechanisms associated with them.

Acknowledgements: This work was supported by the Ministry of Education (Herakleitos II).

PP-12

EFFECT OF PROTON PUMP INHIBITORS ON THE PYLORIC SPHINCTER OF THE RABBIT

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BACKGROUND & OBJECTIVES

Failure of proton pump inhibitors (PPIs) in effectively treating gastro-esophageal reflux disease (GERD) is due to continuing non-acidic reflux, attributed either to the relaxing effect of these agents on the lower esophageal sphincter tone, or to the delayed gastric emptying. Considering that the pyloric sphincter (PS) contractile activity plays an important role in gastric emptying, this study aimed to investigate the pharmacodynamic effects of omeprazole and lansoprazole, two currently prescribed PPIs, on the spontaneous contractile activity of the PS of the rabbit.

METHODS

Following careful removal of the mucosa, rabbit PS strips were prepared and placed in organ baths containing warmed (37°C) and gassed (95%O_2-5%CO_2) Krebs solution. Full concentration-response curves of both PPIs were constructed.

RESULTS

The PS strips developed spontaneous phasic contractions. Both PPIs tested, at concentrations ranging from 10^{-5} to 10^{-3} M, produced only a very small decrease in the basal tone, while they considerably inhibited both the amplitude and frequency of phasic contractions; the latter were completely suppressed at PPIs concentrations higher than 10^{-3} M.

CONCLUSIONS

Gastric emptying is a highly synchronized process involving the integration of the propulsive forces of the antrum contractions and the inhibitory ones of the pyloric muscle. In view of this, the here observed inhibitory effect of PPIs on the PS contractile activity may lead to reduced pyloric resistance and to rather facilitate than delay gastric emptying. Conclusively, the PS response to PPIs does not seem to contribute to the continuing reflux observed in a proportion of GERD patients undergoing PPIs treatment.

PP-13

NOVEL PYRAZOLOPYRIDINES DERIVATIVES AS POTENTIAL ANTI-ANGIOGENIC AGENTS

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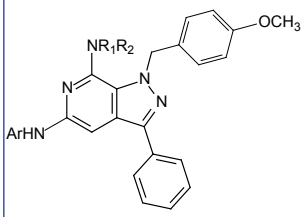
BACKGROUND & OBJECTIVES

Receptor and non-receptor tyrosine kinases (TK) are essential regulators of normal cell homeostasis. Dysregulation of these signalling mediators is often associated with serious clinical disorders including cancer. Inhibitors of certain TK members, such as vascular endothelial growth factor receptors (VEGFR) which are known to promote tumour-induced angiogenesis, have been successfully used in cancer therapy. Since quinazoline and pyridopyrimidine derivatives have been proved to be potent and highly selective inhibitors of TK, in this work we aimed to synthesize novel substituted pyrazolopyridines and explore their capability to modulate crucial stages of the angiogenesis process.

METHODS

Compounds of the general structure shown below:



were synthesized using 2-amino-5-nitro-4-picoline as starting material, which upon suitable transformations was converted to 5-chloropyrazolo[3,4-c]pyridine. A 4-methoxybenzyl group was inserted at N1 of the heterocyclic ring system followed by the addition of a 3-phenyl group. A second chlorine atom was then introduced at position 7 and then both chlorine atoms were successively displaced by suitable amines.
The biological effects of these compounds on the angiogenic phenotype of primary human umbilical vein endothelial cells (HUVEC) were investigated using established in vitro assays of cell proliferation (MTT), migration (transwell) and differentiation into tube-like structures (Matrigel).

RESULTS

Amongst test compounds, three were able to significantly inhibit HUVEC growth, chemotactic migration and tube-like formation in a concentration-dependent manner, though with a diverse efficiency.

CONCLUSIONS

Overall, three novel pyrazolopyridines derivatives with promising anti-angiogenic activities were identified. Analysis of structure-function relationships allowed detection of optimal structural requirements for anti-angiogenic activity.

PP-14

DIFFERENT PRODUCTION OF HYDROGEN SULFIDE (H2S) IN ACUTE AND CHRONIC EXPERIMENTAL COLITIS

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BACKGROUND & OBJECTIVES

Ulcerative colitis (UC) is a chronic Inflammatory Bowel Disease (IBD), with increasing incidence particularly in younger ages, and significant impact in the quality of life of the affected individuals. From recent studies it becomes evident that miscommunication between gut microbiome and the host innate immunity represents a major part in the pathogenesis of the disease. Genetics is implicated by affecting the susceptibility, although it has not been shown to act in a predominant fashion. Because of the complex nature and progress of the disease, treatment choices remain limited. Further understanding of its pathophysiology may lead to the identification of more specific and efficient treatment regimens. Hydrogen sulfide (H_2S) is a water- soluble gas produced in many mammalian tissues, including gastrointestinal tract, through metabolism of L-cysteine by enzymes including cystathionine β- synthase (CBS), cystathionine γ- lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MPST). It has been shown that H_2S can impact on the development of inflammation, whereas it has been reported to accelerate healing of injured gastrointestinal tissue. Our aim is to characterize the contribution of H_2S in the development of experimental colitis in mice and its specific impact during the inflammatory and repair phases of the disease.

METHODS

We used C56Bl6 male mice and applied an experimental model of innate immunity- dependent ulcerative colitis, the Dextran Sodium Sulfate (DSS) model. DSS was administered in their drinking water and animals were sacrificed in different time points. Clinical signs of the disease, histology and the expression levels of CBS, CSE and 3-MPST enzymes were determined using Q-PCR and Western Blot in the affected tissue. The statistical analysis of the results was done by Two-way and One- way ANOVA.

RESULTS

Expression of all three enzymes, i.e. CBS, CSE and 3-MPST, implicated in H_2S metabolism was detected over the course of the disease, both at the gene and protein levels. We found that the mRNA and protein levels of all enzymes were significantly increased during the first three days after the onset of the disease coinciding with the active inflammatory process in the tissue. interestingly, we detected significantly decreased expression in the phase of tissue repair reflecting the resolution phase of inflammation.

CONCLUSIONS

Our findings indicate a phase of the disease-dependent regulation of CBS, CSE and 3- MPST enzymes at the transrcriptional and translational level. The significance of the specific regulation of H2S production in the inflamed gut in the pathogenesis of UC is under investigation.

PP-15

STRUCTURE AND MOLECULAR MECHANISMS OF ACTIVATION OF CRF1 RECEPTOR

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BACKGROUND & OBJECTIVES

The 41-amino-acid peptide corticotropin releasing factor (CRF) plays a key role in the response of our body to stressful stimuli and the maintenance of homeostasis. These effects are mostly mediated by its type 1 receptor (CRF_1R). Malfunctioning of CRF/CRF_1-neuronal circuits is closely associated with the appearance of anxiety and depression, whereas small non-peptide CRF_1R antagonists have been shown to be effective against these neuropsychiatric disorders. The CRF_1R is a class B G-protein coupled receptor (GPCR), which contains seven alpha helical transmembrane domains (TMs). Here, we aimed to obtain structural information for the third TM (TM3) of the apo state of full length CRF_1R and to elucidate the molecular mechanisms underlying receptor activation. TM3 was selected because in class A receptors this helix has been shown to form key interactions with ligands, other TMs, and G proteins.

METHODS

To accomplish this we used pharmacological, biochemical and computational approaches.

RESULTS

Mutation of Phe203 to various amino-acids affected differentially the ligand binding and signaling properties of CRF_1R.

CONCLUSIONS

Phe203 in TM3 plays an important role in CRF_1R activation, possibly by participating in functionally important inter-TM interactions. Our results also suggest that Phe203 interacts with non-peptide antagonists, interfering in the interaction network between TM3 and the remaining TMs that stabilize the active state of CRF_1R. These results provide insight into the molecular mechanisms underlying CRF_1R activation and also hold great potential for the design of more efficient small non-peptide CRF_1R antagonists with anxiolytic and antidepressant properties.

PP-16

RESISTIVE BREATHING AGGRAVATES ENDOTOXIN- INDUCED LUNG INFLAMMATION: THE ROLE OF SOLUBLE GUANYLYL CYCLASE

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BACKGROUND & OBJECTIVES

During exacerbations of chronic obstructive pulmonary disease (COPD), increased lung inflammation and the accompanying bronchocostriction lead to significant airway narrowing, i.e. resistive breathing (RB). Thus, we investigated the effect of RB in mice with pre-existing lung inflammation by endotoxin inhalation. Since smooth muscle tone is a crucial determinant of bronchocostriction and involves nitric oxide (NO) signaling through activation of the soluble guanylyl cyclase (sGC), we aimed to investigate the expression and downstream signaling of sGC in the context of RB and pre-existing inflammation.

METHODS

C57BL/6 mice were subjected to RB by restricting tracheal surface area to 50% (tracheal banding): 1. Sham operated (controls) and TB mice 2. Mice treated with inhaled LPS pseudomonas aureginosa for 24 hours. 3. Mice treated with inhaled LPS prior TB. After the end of the experiments the lungs were lavaged; the recovered BALF was used to count cells, protein concentration and cytokines. We obtained lung tissue samples for immunoblotting focused on sGC signaling.

RESULTS

Mice subjected to LPS and TB exhibited an increased BALF cellularity, protein content and cytokines concentration, tumor necrosis factor (TNF)-α, interleukine(IL)-1β and IL-6, compared with mice treated with inhaled LPS alone. TB downregulated sGC more in pre-existing lung inflammation compared with LPS mice.

CONCLUSIONS

TB aggravates pre-existing lung inflammation in mice and induces a pronounced downregulation of sGC. Our model of TB might offer new insights by which resistive breathing can enhance inflammation in the obstructive lung disease.

PP-17

CIGARETTE SMOKING ABROGATES THE INFARCT LIMITING EFFECTS OF ISCHEMIC POSTCONDITIONING THROUGH INSUFFICIENT ACTIVATION OF AKT AND eNOS

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BACKGROUND & OBJECTIVES

Cigarette smoking is an established risk factor for cardiovascular disease. Ischemic conditioning decreases myocardial infarct size under physiological conditions, however, comorbidities ameliorate its beneficial effects. We investigated the effects of cigarette smoke (CS) exposure on myocardial infarction and its interference with conditioning mechanisms.

METHODS

C57BL/6 mice were divided in 2 groups: Control (RA; exposed to Room Air) or exposed to CS for 4 weeks. Animals from both groups were further divided into 3 subgroups and subjected to 30 min myocardial isc followed by 2 hours of rep: 1) Control, 2) PreC, 5 min isc-5 min rep applied before sustained isc and 3) PostC, 3 cycles of 10 sec isc-10 sec rep applied immediately after sustained isc. The infarct size and area-at-risk were estimated (%I/R).

RESULTS

CS and RA had similar infarct size (38.3±3.6% vs 40.9±2.2%, p=NS). PreC was beneficial for both CS (12.1±1.6%) and RA (17.9±1.8%), $p<0.05$ vs Control. PostC failed to limit infarct size in CS animals, versus RA mice (43.3±2.2% vs 17.0±2.7%, $p<0.05$). Tissue cGMP concentration, sGC, eNOS, iNOS and p-VASP were increased in CS vs RA. PreC in both groups and PostC in RA group activated AKT, whereas PostC failed to enhance p-AKT in the CS group. Enhanced p-eNOS was evident in preconditioned RA and CS, and PostC RA, but not in PostC CS.

CONCLUSIONS

Exposure to CS does not per se increase infarct size. Cardioprotection by PreC is preserved in CS mice, possibly through activation of Akt/eNOS, while the postconditioning benefit is lost.

PP-19

EFFECTS OF ANESTHETIC KETAMINE ON ANXIETY-LIKE BEHAVIOUR IN RATS

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BACKGROUND & OBJECTIVES

There is poor experimental evidence concerning the effects of anesthetic doses of the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine on anxiety. The present study investigated the effects of anesthetic ketamine (100 mg/kg, i.p.) on anxiety-like behaviour in rats.

METHODS

For this purpose, the light/dark (LD) and the open field (OF) tests were used. The effects of anesthetic ketamine on motility were assessed using a motor activity chamber.

RESULTS

Anesthetic ketamine tested 24 h after treatment induced decreases in the number of transitions between the light and dark chambers and time spent in the light chamber compared to the vehicle-treated animals in the LD test. In the OF test, rats that received anesthetic ketamine spent less time in the periphery and the center of the apparatus compared to their control cohorts. Motor activity of ketamine-treated animals was lower to that displayed by the vehicle-treated rats.
Interestingly, the anesthetic ketamine induced effects on anxiety indices were differentiated following an interval of 48 h post-treatment. The rats displayed an anxiogenic-like profile in the LD procedure but not in the OF test, while ketamine did not influence rats' motor activity.

CONCLUSIONS

The present results indicate that an anesthetic dose of ketamine induced a decrease in motor activity 24 h post-treatment, while produced an anxiogenic-like profile that cannot be attributed to changes in motor activity 48 h post-treatment.

PP-20

THE NOVEL DEHYDROEPIANDROSTERONE (DHEA) ANALOGUE BNN27 COUNTERACTED DELAY-DEPENDENT AND SCOPOLAMINE-INDUCED PERFORMANCE DEFICITS IN A RECOGNITION MEMORY TASK IN RATS

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BACKGROUND & OBJECTIVES

Consistent experimental evidence suggests the involvement of neurosteroid dehydroepiandrosterone (DHEA) in cognition. BNN27 is a novel DHEA analogue, which devoid of steroidogenic activity and its neurotrophic effect has been observed. Its role, however, in learning and memory has not yet established. Thus, the present study was designed to investigate the effects of BNN27 on rats' recognition memory.

METHODS

For this purpose, the novel object recognition task was used.

RESULTS

Post-training administration of BNN27 (3-10 mg/kg, i.p.) reversed extinction of recognition memory in the normal rat. In addition, BNN27 (3 and 10 mg/kg, i.p.) counteracted the scopolamine (0.2 mg/kg, s.c.)-induced performance deficits in this recognition memory task.

CONCLUSIONS

The present results indicate that this novel DHEA analogue may modulates different aspects of recognition memory and suggest that its interaction with the cholinergic system is relevant to cognition.

PP-21

INDEPENDENT AND SYNERGISTIC EFFECTS OF THE MAPT AND SNCA GENES IN PARKINSON'S DISEASE AND SCREENING OF COMPOUNDS FOR THE PREVENTION OF TAU-INDUCED DEFECTS IN DROSOPHILA VISUAL SYSTEM

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BACKGROUND & OBJECTIVES

Interaction of α-synuclein and protein tau has been proposed as a possible mechanism in Parkinson's disease (PD). The utility of the Drosophila system in testing candidate compounds against tau-dependent neuronal dysfunction has been determined.

METHODS

An inversion polymorphism of the microtubule-associated protein tau (*MAPT*) gene defines two haplotypes, H1 and H2. We set out to determine whether a) H1 haplotype, b) SNPs included in H1, c) subhaplotypes within H1 defined by these SNPs, and d) the synergistic interaction between the *MAPT* H1H1 genotype and the SNP rs356219 from the 3' region of *SNCA* (α-synuclein gene), are associated with PD, in patients and control subjects of Greek and Italian origin. We utilized transgenic fruitflies carrying human tau genes expressed under the UAS(upstream activator sequence)/Gal4 system to investigate the ability of candidate compounds to inhibit tau-mediated degeneration of photoreceptor neurons.

RESULTS

Overall the frequency of the H1H1 genotype was 63.35 % in PD cases and 56.6% in controls (OR: 1.33, 95% CI: 0.98-1.79). There was no significant difference between cases and control subjects in the overall frequency distribution of H1 SNPs, H1 subhaplotypes, SNCA rs356219 GG marker and its combination with MAPT H1H1 genotype. A 1,2,3-dithiazole compound attenuated the disruption of the regular array of ommatidia in Drosophila eye, but this result could not be replicated.

CONCLUSIONS

Our data show strong evidence of an association between the H1H1 genotype and PD. The finding from the present study might constitute a basis for the design of new compounds with improved activity in repressing transgenic tau in a Drosophila model.

PP-22

TETRACYCLINE ANTIBIOTICS AS INHIBITORS OF H2S SYNTHESIS

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BACKGROUND & OBJECTIVES

Hydrogen sulfide (H_2S) has emerged as a gaseous signaling molecule with important roles in neuromodulation, blood pressure regulation, angiogenesis, cellular energetics and apoptosis. H_2S is produced endogenously by three enzymes in the sulfur metabolic network, namely cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST). Although several compounds that inhibit CSE are available, no selective inhibitors for CBS or 3MST currently exist. In the present study we screened the LOPAC library for inhibitors of H_2S-producing enzymes.

METHODS

The methylene blue assay was used to measure H_2S production from recombinant enzymes. HCT116 and HT29 cells were used for MTT-based proliferation assays.

RESULTS

Screening of the LOPAC library yielded several hit compounds that blocked H_2S-synthesizing enzymes to varying extents. Among those, doxycycline and demeclocycline were found to inhibit 3MST by approximately 50% at 100 uM, with doxycycline being a more potent 3MST inhibitor than demeclocycline in the *in vitro* activity assay. Tetracycline, meclocycline, oxytetracycline, methacycline, tigecycline and minocycline did not affect 3MST activity when used up to 300 uM. From the above-mentioned tetracyclines, tigecycline at 100 uM caused only a slight inhibition of both CBS and CSE; tigecycline additionally inhibited CSE. In a cell-based assay, demeclocycline, but not doxycycline, inhibited the growth of HCT116 and HT29, two colon cancer cell lines in which CBS is highly expressed and H_2S supports cell proliferation.

CONCLUSIONS

We conclude that based on its selectivity towards 3MST, demeclocycline could serve as a lead compound that after undergoing optimization could yield future selective 3MST inhibitors.

PP-23

ASSOCIATION OF COL5A1, MMP3, COL1A1 GENES WITH INJURIES DURING SPORTS ACTIVITY IN A GREEK POPULATION OF 739 SUBJECTS

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BACKGROUND & OBJECTIVES

Tendon and ligaments within the upper and lower limbs are some of the more common sites of musculoskeletal injuries during physical activity. Several extrinsic and intrinsic factors have been shown to be associated with these injuries. More recently, studies have suggested that there is also, at least in part, a genetic component to the Achilles tendon (AT) and anterior cruciate ligament (ACL) injuries. Sequence variants of the COL5A1, MMP3 and COL1A1 genes showed association with Achilles tendinopathies and ACL ruptures. Those genes encode for proteins directly involved in biological processes within tendons and ligaments.

METHODS

In this genetic association study, the 739 recruited Greek participants consisted of 378 females (51,2%) and 361 males (48,8%) aged from 1 to 94 years. The participants were genotyped for the COL5A1 rs12722 (C/T), MMP3 rs679620 (A/G) and COL1A1 rs1800012 (G/T) sequence variants. The cohort was divided into 7 subgroups according to the SNP or/and the SNPs combination examined.

RESULTS

The vast majority of genotype combinations appeared to have a different impact on the risk of each injury. The genotype combination CT+AG+GG, (over-represented among our population within the group VII, 14,9%), was associated with increased risk of ACL rupture (GS=4), although it tended to have a protective effect against Achilles tendinopathy (GS=1). Furthermore, within the same group, the protective CC+AG+TT_{1A1} combination and the hazardous TT_{5A1}+GG+GG_{1A1} combination were underrepresented (0,3% and 5,8%, respectively). Similar findings were identified within the other 6 subgroups.

CONCLUSIONS

This study demonstrated that chronic Achilles tendinopathy and ACL ruptures are distinctly different injuries with genetic predisposition. Only a minority of the Greek population examined seemed to be protected for both injuries. Finally, individuals at increased risk of both ATP and ACL ruptures were underrepresented among the cohort.

PP-24

INCIDENCE OF SINGLE NUCLEOTIDE POLYMORPHISM Rs9923231 IN THE VKORC1 GENE, Rs1799853 AND Rs1057910 IN THE CYP2C9 GENE ASSOCIATED WITH WARFARIN, ON A 614 GREEK VOLUNTEER SAMPLE

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BACKGROUND AND OBJECTIVES

We studied the incidence of SNPs in the VKORC1 and CYP2C9 genes associated with warfarin, in a Greek population. The VKORC1 gene is responsible for the organism's response to warfarin and CYP2C9 is involved in its metabolism.

METHODS

614 people from the general population took part. Epithelial cells from the the participants' oral cavity were taken followed by DNA extraction. Genome analysis of all study subjects for the presence of VKORC1 and CYP2C9 polymorphisms was performed by Real Time PCR.

RESULTS

For the wild-type allele (CC genotype) of Rs9923231, 26,4% of the volunteers were homozygous, 46,2% heterozygous (CT) and 27,4% homozygous for the mutant allele (TT). For Rs1799853, 73% were homozygous for the wild-type allele (CC), 24,8% were heterozygous (CT), and 2,3% were homozygous for the mutant allele (TT) and for Rs1057910, 85% were homozygous for the wild-type allele (AA), 14,7% heterozygous (AC) and 0,3% homozygous for the mutant allele (CC). Statistical analysis showed that in heterozygous and even more in homozygous individuals for the Rs9923231 polymorphism of the VKORC1 gene, the average daily requirements for warfarin are less compared to individuals lacking VKORC1 mutations. Individuals with CYP2C9 Rs1799853 and Rs1057910 polymorphisms, have reduced enzyme activity, metabolize warfarin slowler and thus may require lower doses of warfarin than those not having these polymorphisms.

CONCLUSIONS

Studying the patients' genotype, individualization of warfarin administered dose is enabled, in order to be therapeutically effective and safe, with limited risk of toxicity and adverse reactions.

PP-25

GENE POLYMORPHISMS PREDISPOSING TO TYPE II DIABETES MELLITUS AND/OR OBESITY IN 513 GREEKS: FREQUENCY DISTRIBUTION OF TCF7L2, MTNR1B, CDKAL1, SLC30A8 AND FTO

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BACKGROUND AND OBJECTIVES

Diabetes mellitus type 2 is a chronic metabolic disease with pandemic spread worldwide. Environmental influences and genetic background are included in the pathogenesis of the disease. More than 40 suspected loci have been associated with the risk of developing type 2 diabetes. Four of these (rs7903146, rs10830963, rs7756992, rs13266634) were studied in a sample of Greek population. Obesity is also a chronic disease and is the most important risk factor for type 2 diabetes. Many SNPs have been associated with obesity with predominant the FTO rs9939609, which has been studied in the same sample.

METHODS

For the present study were studied 513 volunteers. The DNA used was collected from buccal epithelial cells of the oral cavity of the volunteer. It was isolated by the extraction method and measured by the method of Real Time PCR.

RESULTS

The results have shown that the frequency of alleged dangerous alleles is ranging from 25% to 70%. There were no differences in the frequencies between both sexes in genotypes and allele level. The rs9939609 is quite frequent (44% frequency of A), without any differences in both sexes and has also been associated with type 2 diabetes, although it is still unknown whether the association is independent or not by obesity.

CONCLUSIONS

Although the genetic basis of type 2 diabetes and obesity is now proven, it is not yet unfortunately been transferred in daily clinical practice. It is, however, expected in the following years to contribute significantly to the prevention, diagnosis and treatment of these frequent diseases.

PP-26

ANALYSIS OF POLYMORPHISMS RS2476601, RS3761847, RS660895 AND RS7574865 INVOLVED IN CLINICAL AND IMMUNOLOGICAL MANIFESTATIONS OF RHEUMATOID ARTHRITIS IN 472 INDIVIDUALS.

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BACKGROUND & OBJECTIVES

Rheumatoid Arthritis is a multifactorial disease, the possible genetic components of which are continuously and systematically researched. The discovery of SNPs (Single Nucleotide Polymorphism,) by the addition, deletion or change of a base have led to the discovery of new genetic correlations with the disease. The objective of this particular study is to demonstrate how the polymorphisms in specific loci RS2476601, RS3761847, RS660895 and RS7574865 affect various clinical and immunological manifestations of RA.

METHODS

The analysis was carried out on a sample of 472 healthy individuals from Greece. Genomic DNA used was isolated from the epithelial cells of the oral cavity and analyzed by Real-Time Polymerase Chain Reaction.

RESULTS

The genotype of GA RS660895 is 17.37% of the sample, while the GG is the 2.12%. The RS3761847 occurring in 11, 44% as GG genotype and 44.91% as GA .While RS2476601 is 8.26% having a GA genotype and 0.21% having an AA. The homozygous RS7574865 as TT is 6.57%, while the GT is 39.40%.

CONCLUSION

All 4 polymorphisms were found in the Greek population, however, RS3761847 alongside RS7574865 show higher MAF (Minor Allele Frequency) and may be responsible for increased susceptibility to RA.

PP-27

GENETIC SUSCEPTIBILITY BIOMARKERS OF ALZHEIMER'S DISEASE DEVELOPMENT: THE ROLE OF RS7412 AND RS429358 POLYMORPHISMS

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BACKGROUND & OBJECTIVES

Alzheimer's disease is a degenerative disease of the central nervous system characterized by loss of memory, difficulty in executing daily tasks, anxiety, aggression and at the final stages the patient falls in coma and death occurs. In this study the polymorphisms rs7412 and rs429358of apolipoprotein E (ApoE) gene have been analyzed, to reveal their relationship with Alzheimer's disease, separately and combined on the same allele.

METHODS

DNA from 95 cases, with confirmed AD diagnosis, and 100 non affected controls, was isolated by standard DNA extraction and real time Polymerase Chain Reaction was applied to detect the polymorphisms in all subjects. Statistical analysis was performed and in order to determinate possible genetic disorder that predisposes to AD the odds ratios (OR) and 95% confidence intervals (95%CI) of each polymorphism between cases and controls were evaluated. Further, comparison between male and female within the AD patients was carried out, to reveal differences in the susceptibility between genders.

RESULTS

In this study OR of polymorphism rs7412, alone, in the Alzheimer population, in male and in female, were 0.69, 0.61 and 0.85 respectively, as compared to age matched controls. The OR rs429358 polymorphism, alone, in the Alzheimer group, in male and in female patients were 3.78, 2.91 and 5.90, respectively, as compared to aged matched controls.

CONCLUSIONS

The contribution of ApoE in the development of AD was confirmed with the exception of the polymorphism rs7412 when not combined with other polymorphisms. The polymorphism rs429358 alone or in combination with rs7412 on the same allele predisposes male to Alzheimer's disease twice as much as compared to females.

PP-28

HOW DO GENES INFLUENCE PERSONALITY: A RESEARCH IN 830 GREEK SUBJECTS.

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BACKGROUND & OBJECTIVES

Intelligence consists of a series of competencies (e.g. perception, learning, adaptation). Decision making is defined as the conscious choice between given options, relating to a problem. The genetic background and the environment are the key elements for the personality characteristics of the human being. The object of this study is to determine the rs324420, rs1800497, rs363050, rs6265, rs1328674 gene effect on these topics, in 830 Greek Subjects.

METHODS

The population of the volunteers is described, based on genotype, sex, with the respective frequencies, including the Minor Allele Frequency (MAF). A potential relationship of the volunteer genus with the above characteristics is checked and finally, volunteers are classified; a volunteer receives +1/-1, for each genotype and haplotype, which enhances/relegates his intelligence or his decision-making respectively.

RESULTS

No statistically significant gender-characteristics correlation is observed. At a rate of 92.5%, the volunteers are characterized by prudence and temperance of thought, with only a small proportion of them (7.5%) being genetically spontaneous and adventurous. Regarding intelligence, the population is around an average and a little above it, at a rate of 96.3%, while the edges of the scale suggest only a 0.5% of the volunteers, who, although the "smartest", somehow lack prudence.

CONCLUSIONS

Individuals with low cognitive ability may be more prudent than others and vice versa, while the "smartest" ones tend to be more risky, in decision-making. Therefore, intelligence and decision-making, after all, may be less linked to each other than expected. These results may be useful in targeted and personalized therapies of relevant diseases.

PP-29

LOW-FLOW HIGH-OZONE CONCENTRATION DISINFECTION OF DENTAL IMPRESSIONS

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BACKGROUND & OBJECTIVES

The disinfection of dental impression materials to ensure no cross-contamination from potential pathogenic bacteria of oral microflora is conducted in Dental practice via chemical disinfectants. Low flow-high ozone concentration disinfection was used, by means of a prototype device, aiming in elimination of impression material dimensional change problems that current chemical disinfection faces.

METHODS

Disc shaped dental addition-cured silicone was inoculated with *Klebsiella pneumoniae* and *Staphylococcus aureus*, 10mm discs were removed and ozone disinfected for different time intervals, immersion disinfected or served as controls. Disinfection success was examined by using the viable plate count method, while the statistical analysis was conducted via one way-ANOVA ($p < 0.05$). 120 dental elastomeric impression material specimens (4 material groups) were fabricated according to the ISO 4823 guidelines. Measurements regarding their linear dimensional stability were taken using a measuring microscope before and after ozone disinfection, sodium hypochlorite solution immersion disinfection (0,525%) and benzalkonium chloride solution immersion disinfection (0,3%).

RESULTS

Significant eradication was observed for selected Gram (+) and Gram (-) bacteria after 3 minutes of ozone exposure, leading to complete disinfection of the samples. The lowest linear dimensional changes were found on the ozone disinfected samples which were statistically non-significant.

CONCLUSIONS

While immersion disinfection of dental impressions is currently the most widely accepted method of disinfecting dental impressions, low-flow high-ozone concentration disinfection provides a quick, efficient, fully automated alternative method, limiting liquid waste generation. The dimensional stability provided designates this disinfection method highly innovative in the Prosthetic Dentistry field.. A precise automated method for impression disinfection is established, relieving the dental team of possible cross-contamination.

PP-30

BREAST CANCER AND GENETIC ASSOCIATION: A STUDY AMONG 513 NON-AFFECTED WOMEN OF GREEK ORIGIN.

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BACKGROUND & OBJECTIVES

Its is well documented that individuals may have increased risk to develop breast cancer (BRCA), as compared to the general female population, because of the existence of inherited gene variants. In this study we examined BRCA1 and BRCA2 tumor suppressor gene polymorphisms (RS1799950, 185delAG, 5331GA, 5382insC, R1751X, RS144848, G4X and 617delT), that may cause deregulation, leading to uncontrolled cell proliferation and tumor development. Further, genes encoding TNFa and Il6, as indicators of inflammation, expressing angiogenic and oncogenic properties, the CHEK2 polymorphism, RS17879961, that leads in inability to repair DNA damage, the AURKA, RS2273535, polymorphism that is associated with abnormal segregation of chromosomes, the FGFR2 polymorphisms, RS2981582, and RS7965399, and the BRCA associated IGF1 gene polymorphism.

METHODS

DNA extraction from buccal epithelial cells and rtPCR was performed in 513 Greek non affected female subjects (2-91 years). Frequencies of polymorphisms were then calculated. All individuals gave written consent to use DNA data after anonymization.

RESULTS

BRCA1-RS1799950 revealed 79.49% A:A (wt), 19,45% A:G, and 1,06% G:G genotypes. All other BRCA1 polymorphisms examined were wt. BRCA2-RS144848 showed genotypic alteration (G:G) in 5,36% of the population, 54.85% were wt T:T and 39,79% heterozygous G:T. The TNFa frequencies were, mutated A:A, 0.84%, wild-type G:G, 84,10% and heterozygous G:A, 15,06%. Il6, showed 55,04% G:G (wt), 38,45% C:G and 6,51% C:C. CHEK2, was 98,90% T:T (wt) and 1,10% C:T. (No CHEK2-C:C carriers). AURKA, revealed 62,97% A:A, 33,26% T:A and 3,77% T:T (wt). Finally, FGFR2, was 29,30% C:C (wt), 52,56% C:T, 18,14% T:T, and IGF1 was 92,03% T:T (wt), 7,54% C:T and 0,43% C:C.

CONCLUSIONS

BRCA1 and BRCA2 mutations lead to unsuccessful DNA repair. Homozygous mutated, and probably also heterozygous females, may have higher risk in developing BRCA and, possibly, early therapeutic interventions may be more successful, promoting health and enabling family planning.

PP-31

CURRENT THERAPEUTIC APPROACHES IN TRIPLE-NEGATIVE BREAST CANCER

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BACKROUND AND OBJECTIVES

Triple negative breast cancer (TNBC) is an aggressive clinical phenotype characterized by lack of expression (or minimal expression) of estrogen receptor (ER) and progesterone receptor (PR) as well as the absence of human epidermal growth factor receptor–2 (HER2) overexpression. It shows substantial overlap with basal-type and BRCA1-related breast cancers, both of which also have aggressive clinical courses. Because of its expression profile, TNBC is not amenable to treatment with hormone therapy and systemic treatment options are currently limited to cytotoxic chemotherapy. Overall survival, whether in early-stage or advanced disease, is poor compared with that in patients who have other phenotypes. A number of targeted approaches to TNBC are undergoing clinical evaluation, including the use of agents with poly- (ADP-ribose)-polymerase inhibitory properties such as iniparib (BSI-201), olaparib (AZD2281), and veliparib (ABT-888), antiangiogenic agents such as bevacizumab and sunitinib, and epidermal growth factor receptor blockers such as cetuximab and erlotinib.

METHODS

The clinical characteristics of TNBC and clinical experience to date with novel targeted agents under development for this aggressive phenotype is reviewed. The recent literature from PubMed and Medline databases was reviewed.

RESULTS

Encouraging results with some of these agents have been reported, thereby offering the promise for improved outcomes in patients with TNBC. In terms of chemotherapeutic treatment, anthracyclines and taxanes are useful drugs, and high response rates have been described. The combination with antiangiogenic drugs has also proven useful. Poly- (ADP-ribose)-polymerase inhibitors, showed favorable results in TN tumors with BRCA mutation. There are currently several ongoing studies with new drugs including epidermal growth factor receptor inhibitors, c-kit inhibitors, Raf/Mek/Map kinase inhibitors and mTOR inhibitors, that have shown promising results.

CONCLUSIONS

In the absence of approved targeted agents for the treatment of TNBC, most new agents remain experimental. Increased understanding of molecular profiles of TNBC subtypes is likely to improve therapeutic strategies with targeted agents.

PP-33

HYDROGEN SULFIDE DONORS REDUCE MONOCYTE CELL ADHESION TO ENDOTHELIAL MONOLAYER

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BACKGROUND & OBJECTIVES

Inflammation and monocyte cell adhesion to endothelium are two key events in the early stages of atherogenesis. We sought to examine the possible vascular anti-atherosclerotic effect of two H_2S donors, GYY4137 and thioglycine, in an *in vitro* model.

METHODS

EAhy926 cells ($3x10^6$) were grown to confluence in 24-well plates. Cells were serum-starved for 12h and then incubated with several concentrations of GYY4137 or thioglycine for various time points. After H_2S donor treatment, cells were incubated with TNF-α (10ng/ml) for 6h. Medium was then removed, THP-1 monocytes were counted and seeded on TNF-α activated EAhy926 monolayers and incubated for 1h at 37°C. Non-adherent THP-1 cells were removed and EAhy926 were fixed with 4% paraformaldehyde. Adhesion was counted on three randomly selected 40x magnification microscopic fields/well in four independent experiments.

RESULTS

Control-confluent EAhy926 showed minimal binding to THP-1 cells, while the adhesion of THP-1 was significantly increased to TNF-α-stimulated EAhy926. Pre-treatment of EAhy926 with increasing GYY4137 (300μM & 500μM) or thioglycine (100μM, 300μM & 500μM) concentrations for 1h before TNF-α activation caused a concentration dependant inhibition of THP-1 adhesion up to 69.3±2.2 % and 48.3±2.8 %, respectively. Pre-treatment of EAhy926 with glycine under similar conditions did not attenuate the adhesion of THP-1 to EAhy926 cells.

CONCLUSIONS

H_2S exerts a potentially protective role in atherosclerotic pathogenesis by inhibiting monocyte cell adhesion to endothelial cells. Utilization of H_2S donors might, thus, be a new therapeutic strategy against this pathological process.

PP-34

AUTOMODULATION OF VASCULAR HISTAMINE LEVELS BY HISTAMINE RECEPTOR ANTAGONISTS: NOVEL INSIGHTS INTO SYSTEMIC INFLAMMATION

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BACKGROUND & OBJECTIVES

The impact of histamine in a variety of important biological functions has been underscored by the blockbuster success of the H_1R and H_2R antagonists. The identification of H_3R and H_4R revived histamine research and new areas are now being explored. This study aimed to investigate the putative peripheral histamine automodulation in the rat blood vessels.

METHODS

Adjuvant arthritis was induced in male Wistar rats by complete Freund's adjuvant and the animals were scored daily for arthritis signs in the paws. The H_1R, H_3R and H_4R antagonists, dimethindene, GSK334429 and JNJ7777120 were administered intraperitoneally on day 0 (prophylactic model dosing), the respective controls receiving normal saline. Following sacrifice on day 21, the abdominal aorta (AA) and the inferior vena cava (IVC) were dissected out. Tissue histamine content was quantified fluorophotometrically.

RESULTS

Extra-articular inflammation was evidenced by the significant increases in vascular histamine levels in arthritic animals. Contrary to dimethindene, GSK334429 and JNJ7777120 increased dose-dependently the basal histamine levels in normal vessels without inducing arthritic signs. In adjuvant arthritis, dimethindene and GSK334429, but not JNJ7777120, restored basal histamine content in the IVC and AA, respectively.

CONCLUSIONS

The findings provide first evidence for the intrinsic regulation of vascular histamine levels by H_3R and H_4R and for the differential involvement of H_1R and H_3R in arthritis. The related feedback mechanisms underlying peripheral histamine regulation in a range of poorly treatable chronic inflammatory diseases are currently under investigation.

This work was part of the EU FP7 COST BM0806

PP-35

K_{ATP} CHANNEL ACTIVATION INDUCES ANGIOGENESIS IN VITRO AND IN VIVO

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BACKGROUND AND OBJECTIVES

The angiogenic effects of hydrogen sulfide depend on the activation of K_{ATP} channels. In addition, it has been shown that C-type natriuretic peptide (CNP) induces growth responses in endothelial cells.
Our aim was to test a) whether direct pharmacological K_{ATP} channel activation or blockade modulates angiogenic-type responses, and b) whether the C-type natriuretic peptide (CNP) affects angiogenesis and if it does, whether this depends on K_{ATP} channel activation.

METHODS

The chicken chorioallantoic membrane (CAM) assay was used as *in vivo* angiogenesis model. Human Umbilical Vein Endothelial cells (HUVECs) and the murine brain endothelial cell line bEnd.3 were used *in vitro* in Matrigel, proliferation and migration assays.

RESULTS

Treatment of CAM with either the K_{ATP} channel opener SG-209 or with CNP induced robust angiogenesis that was abolished by the K_{ATP} channel inhibitors Glibenclamide and 5-HD. *In vitro*, the K_{ATP} channel openers SG-209 and Nicorandil increased cell proliferation, cell migration and Matrigel tube-like formation in both bEnd.3 and in HUVECs. These effects were blocked by K_{ATP} channel inhibition or by p38 and ERK1/2 inhibition. In HUVECs, Glibenclamide or knock-down of the K_{ATP} channel subunit Kir6.1 by siRNA abolished the migratory response to SG-209. Similarly, the effect of CNP on HUVEC Matrigel tube-like formation was blocked by introduction of Kir6.1 subunit siRNA.

CONCLUSIONS

- Pharmacological activtion of K_{ATP} channel activation elicits angiogenic responses *in vivo* and *in vitro*.
- The vasoactive peptide CNP displays angiogenic properties that depend on K_{ATP} channel activation.

PP-36

CELLULAR STRESS RESPONSE AND AGEING: THE BUDDING YEAST AS AN EXPERIMENTAL MODEL

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BACKGROUND & OBJECTIVES

The budding yeast *Saccharomyces cerevisiae* is a versatile and powerful model for the investigation of mechanisms underlying the multicomponent cellular stress response (SR) and ageing in eukaryotes. Despite the extensive related literature, data on the identification and functional annotation of the key components that can be modulated by drugs are limited and largely inconclusive. This study investigated the effects of histamine on the SR and ageing in yeast, thereby exploring its potential functions in a histamine receptor-free experimental model and stimulating research on exploitation prospects.

METHODS

Cell growth and viability was determined in cultures grown through to post-logarithmic phase at 27°C. Thermal preconditioning and heat shock were performed by shifting cells to 37°C for 2h and 53°C for 30min, respectively. The effects of pharmacologically active agents were investigated following short- and long-term administration at a range of doses and time points during growth. Ageing was evaluated by the capacity and rate of cell proliferation, survival and morphology in various stages of development.

RESULTS

Histamine failed to induce any alteration in yeast proliferation and survival under normal conditions at any phase of growth. Contrary to the long-term administration, short-term histamine treatment resulted in a dose-dependent induction of the thermotolerant phenotype involving alterations in heat shock protein expression and cytoskeletal components.

CONCLUSIONS

The findings provide evidence for the non-receptor-mediated role of histamine to condition physiological signals in yeast, thus offering the lead for the elucidation of mechanisms underlying the evolutionary-conserved adaptive and protective processes in eukaryotic cells.

PP-37

A STUDY OF THE ASSOCIATION OF COMMON ABCB1 POLYMORPHISMS WITH RESPONSE TO ANTIPSYCHOTIC THERAPY IN A NATURALISTIC SETTING. PRELIMINARY RESULTS

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BACKGROUND & OBJECTIVES

Individual response rates to antipsychotic drugs are subject to wide variation which may be due to genetic factors, among others. P-glycoprotein (P-gp) is an important efflux pump which could affect the intracerebral concentration of many antipsychotic drugs, by virtue of its localization on the blood-brain barrier. Common polymorphisms of the P-gp – coding gene (*ABCB1*) have been associated in the past with response to antipsychotic therapy. Here we report preliminary results from a study of the association of the rs2032582 (2677G>T/A) and rs1045642 (3435C>T) *ABCB1* gene polymorphisms with response to combination therapy of Greek schizophrenic patients.

METHODS

The study is undertaken in its natural setting and involves the administration of both first and second generation antipsychotic drugs to consecutive patients of the 2nd Department of Psychiatry of our Medical School. Dosages are normalized to chlorpromazine equivalents. Genotyping is done with previously published PCR-RFLP methods. The χ^2 test of independence and ANCOVA tests are used for statistical analysis.

RESULTS

So far, no statistically significant differences are detected between initial responders and non-responders (defined as 30% change in the total PANSS score, from baseline to the end of the trial), with respect the genotype distribution of either polymorphism. Patients homozygous for the G allele of rs2032582 appear to receive higher - on average - doses of chlorpromazine equivalents, but that tendency is still non-significant.

CONCLUSIONS

rs2032582 GG carriers could be more refractory to antipsychotic drug therapy but it is too early for definite conclusions to be drawn.

PP-39

H_2S COMPENSATES NO DEFICIENCY IN MURINE CORPUS CAVERNOSUM

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BACKGROUND & OBJECTIVES,

Recent studies show that nitric oxide (NO) is required for the exogenous hydrogen sulfide (H_2S) induced vascular relaxation in rat aorta (Coletta et al., 2012) and human CC (d'Emmanuele di Villa Bianca et al., 2009). However it is unknown, if the endogenous H_2S production and the related relaxation are compensated in the absence of NO.

METHODS

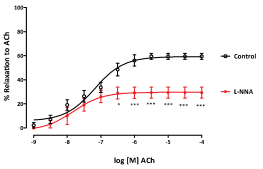
The relaxation responses of murine corpus cavernosum (CC) to ACh, L-Cysteine or sodium hydrogen sulphide (NaHS) were evaluated in presence or absence of NOS inhibitor Nω-Nitro-L-arginine (L-NNA) by using a myograph in organ bath experiments. H_2S production was assessed by a colorimetric assay. Basal H_2S production and the expression level of H_2S producing enzymes were measured in eNOS-/- mice CC, resembling pathologies in erectile dysfunction (ED) and endothelial dysfunction.

RESULTS

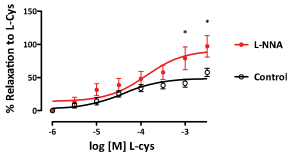
L-NNA increased the concentration-dependent responses to L-cysteine (the substrate for H_2S production) and did not change the relaxation responses to NaHS (a donor of H_2S), in mouse CC strips. Furthermore, L-NNA increased the endogenous H_2S production by L-cysteine in mouse CC. The producing enzymes of H_2S, CSE and 3-MST, showed an increased expression levels in the genetic ablation of eNOS mouse CC.

CONCLUSIONS

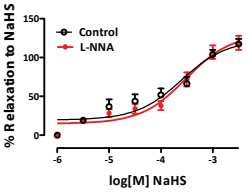
Our findings suggest that H_2S can play a compensatory role in presence of endothelial dysfunction in mouse CC.



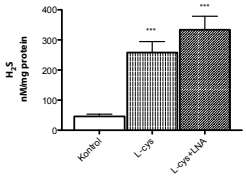
L-NNA inhibits ACh-induced relaxations in mice CC
***P<0.001 Control vs L-NNA (n=6 and 4; respectively, 2way Anova, Bonferroni post hoc test)



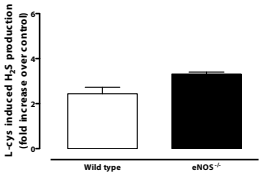
L-NNA increase L-cysteine-induced relaxations in mice CC.
*P<0.05 Control vs L-NNA (n=6 and 4; respectively, 2way Anova, Bonferroni post hoc test)



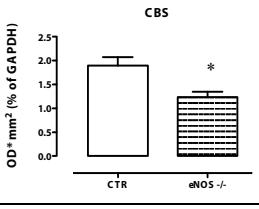
L-NNA does not change NaHS-induced relaxations in mice CC.
P>0.05, ns., Control vs L-NNA (n=6 and 4; respectively, 2way Anova, Bonferroni post hoc test)

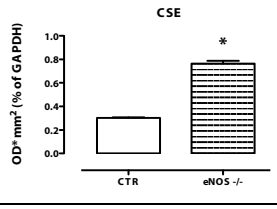


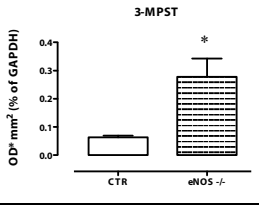
L-NNA increase L-cystein induced H2S production, p<0.001, L –cyst vs L-Cyst+L-NNA, Paired t test, n=18

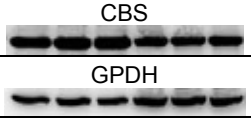


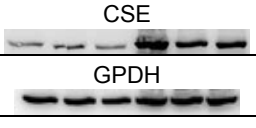
Genetic ablation of eNOS increase L-cystein induced H2S production in mice CC (n=3,2) (values are given as fold increase of basal H2S production)

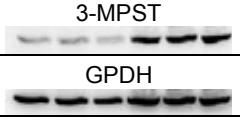












PP-40

THE EFFECT OF HYALURONIC ACID ON WOUND HEALING DEPENDS ON ITS MOLECULAR MASS AND INVOLVES MMP-2

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BACKGROUND & OBJECTIVES

Hyaluronic acid (HA) is an extracellular matrix molecule that regulates important cell functions, such as proliferation and migration and plays an important role in regenerative processes. In this study, we investigated the effect of low and of high molecular weight HA (LMWHA and HMWHA) on wound healing and the involvement of matrix metalloproteinase (MMP) -2 in this process.

METHODS

We used primary cultures of human dermal fibroblasts, and we assessed the effect of HA (small fragments, 50, 250 and 2500 kDa) on wound healing using the scratch wound assay. We also evaluated the effect of 4-methyl-umbeliferone (4-MU), an inhibitor of HA synthases and aristolochic acid (AA-I), an inhibitor of hyaluronidases, on wound healing. Gelatinolytic activity of MMP-2 was assessed by gelatin zymography. Cell viability and proliferation were estimated by cell counting.

RESULTS

LMWHA induced the wound healing process in a dose-depended manner. The same results were also obtained in the presence of 4-MU. On the contrary, HMWHA inhibited wound healing, in a dose-dependent manner and the same results were also obtained in the presence of AA-I. Gelatinolytic activity was significantly increased by LMWHA, but not by HMWHA.

CONCLUSIONS

Our results indicate that the effect of HA on wound healing depends on the size of HA and correlates with increased gelatinolytic activity. The ability of LMWHA to promote wound healing suggests that it could be used as a pharmacological target for the treatment of delayed or complicated wound healing.

PP-41

INTERACTIONS OF THE KAPPA OPIOID RECEPTOR WITH REGULATORS OF G PROTEIN SIGNALLING

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BACKGROUND & OBJECTIVES

Opioid receptors (OR) μ, δ, and κ couple to Gi/Go proteins to modulate a variety of physiological responses in the nervous system. Apart from G proteins, opioid receptor activity is also controlled by interactions with other proteins that regulate their fine tuning opioid [1]. Regulators of G protein Signalling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits and modulate G protein coupled receptor (GPCR) signalling. Previous studies have shown that RGS4 associates with the C-termini of μ- and δ-opioid receptors (μ-OR, δ-OR) in living cells and plays a key role in Gi/Go protein coupling selectivity and signaling of these receptors. [2,3]

METHODS AND RESULTS

To deduce whether similar effect also occurs for the kappa opioid receptor (κ-OR) and define the ability of members of B/R4-RGS family to interact with the kappa opioid receptor (κ-OR) we performed pulldown experiments using GST fusion peptides encompassing the carboxyl terminus of κ-OR. These experiments indicated that RGS2 and RGS4 interact within this receptor subdomain. Co-immunoprecipitation studies indicated that RGS2 and RGS4 associate with κ-OR constitutively and upon receptor activation and confer selectivity for coupling with a specific subset of G proteins. Functional assays have shown that both members of B/R4-RGS family attenuate κ-OR mediated inhibition of adenylyl cyclase and ERK1,2 phosphorylation, but present different amplitude in their effect.

CONCLUSIONS

Our results demonstrate that RGS2 and RGS4 are new interacting partners and negative modulators of κ-OR signaling.

This work was supported by the EU grant «Normolife» (LSHC-CT2006-037733) and the GSRT.

References

[1] Georgoussi, Z., Georganta, E.-M., Milligan, G. (2012) Current Drug Targets, 13, 80-102
[2] Georgoussi Z., Leontiadis L., Mazarakou G., Merkouris M., Hyde K. and Hamm H. (2006), Cell. Signal.18, 771–782
[3] Leontiadis, L. J., Papakonstantinou, M. P., Georgoussi Z. (2009) Cell. Signal. 21, 1218–1228.

PP-42

CYCLIN-DEPENDENT KINASE 5 IS INVOLVED IN PLEIOTROPHIN-INDUCED ENDOTHELIAL CELL MIGRATION

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BACKGROUND & OBJECTIVES

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that requires the regulatory subunits p35 or p39 for activation. CDK5 plays an important role in neuronal migration and neurite outgrowth and there are studies showing its implication in tumor growth and angiogenesis. Pleiotrophin (PTN) is a heparin-binding growth factor that induces cell migration in neuronal, cancer and endothelial cells through its receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ) and $\alpha_v\beta_3$ integrin. In the present study, we investigated the role of CDK5 in PTN-induced endothelial cell migration.

METHODS

Human umbilical vein endothelial cells were used. CDK5 activity was estimated by either direct measurement of the kinase activity or through its interaction with p35. Migration assays were performed in 24-well microchemotaxis chambers. RPTPβ/ζ was suppressed using small interfering RNA. Protein-protein interactions were studied by immunoprecipitation followed by Western blot or MALDI-TOF analyses, or by proximity ligation assays. Statistical analysis was performed by unpaired t-test.

Results

Pharmacological inhibition, as well as down-regulation of CDK5 by siRNA, completely abolished PTN-induced endothelial cell migration. PTN increased CDK5 kinase activity and its interaction with p35. PTN-induced CDK5 activation seemed to be independent of $\alpha_v\beta_3$ but dependent of RPTPβ/ζ expression. Interestingly, both CDK5 and p35 were found to interact with RPTPβ/ζ. Activation of c-Src kinase was involved in CDK5 activation, while pharmacological inhibition of CDK5 did not affect PTN-induced β_3 Tyr773 phosphorylation and ERK1/2 activation.

CONCLUSION

CDK5 is a significant regulator of the PTN/RPTPβ/ζ signaling pathway that contributes to PTN-induced endothelial cell migration.

Acknowledgement: We thank the Advanced Light Microscopy facility of the School of Health Sciences, University of Patras, for using the Leica SP5 confocal microscope.

PP-44

RECEPTOR PROTEIN TYROSINE PHOSPHATASE BETA/ZETA IS INVOLVED IN VASCULAR ENDOTHELIAL GROWTH FACTOR-INDUCED ENDOTHELIAL CELL MIGRATION

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BACKGROUND & OBJECTIVES

Receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) is a transmembrane chondroitin sulfate protein tyrosine phosphatase that acts as a receptor for the growth factor pleiotrophin (PTN). RPTPβ/ζ interacts with $\alpha_v\beta_3$ on the cell surface and upon binding of PTN leads to c-Src Tyr530 dephosphorylation, β_3 integrin Tyr773 phosphorylation, cell surface nucleolin (NCL) localization and stimulation of cell migration. c-Src-mediated β_3Tyr773 phosphorylation is also observed after vascular endothelial growth factor 165 ($VEGF_{165}$) stimulation of endothelial cells and is essential for VEGF receptor 2 (VEGFR2)-β_3 integrin association and subsequent signaling. In this work we studied whether RPTPβ/ζ is involved in VEGF actions.

METHODS

Human umbilical vein endothelial, human glioma M059K and U87MG, and chinese hamster ovary cells were used. Migration assays were performed in 24-well microchemotaxis chambers. RPTPβ/ζ was suppressed using siRNA. Protein-protein interactions were studied by immunoprecipitation/Western blot, immunofluorescence or proximity ligation assays. Statistical analysis was performed by unpaired t-test.

RESULTS

RPTPβ/ζ mediates $VEGF_{165}$-induced c-Src-dependent β_3 Tyr773 phosphorylation, required for both $VEGF_{165}$-induced VEGFR2-$\alpha_v\beta_3$ interaction and the downstream PI3K activation and cell surface NCL localization. By using immunoprecipitation and proximity ligation assays, RPTPβ/ζ was found not to interact with VEGFR2 but to interact with VEGF. This interaction was interrupted by chondroitin sulphate E (CS-E) and PTN but not bevacizumab. CS-E and down-regulation of RPTPβ/ζ by siRNA inhibited $VEGF_{165}$-induced endothelial cell migration, while PTN decreased the migratory effect of $VEGF_{165}$ to the levels of its own effect.

CONCLUSIONS

RPTPβ/ζ is potentially a good target for development of additive or alternative anti-VEGF therapies.

Acknowledgements: This work was supported by the European Union (European Social Fund – ESF, Heracleitus II (M. Koutsioumpa - E. Papadimitriou), Thales (V. Megalooikonomou) and IKY Fellowships of Excellence for Postgraduate studies in Greece - Siemens Program (E. Pantazaka). The authors thank the Advanced Light Microscopy facility of the Medical School, University of Patras for using the Leica SP5 confocal microscope.

PP-45

PLEIOTROPHIN-INDUCED CELL MIGRATION INVOLVES GENERATION OF REACTIVE OXYGEN SPECIES

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BACKGROUND & OBJECTIVES

Pleiotrophin (PTN) is an 18 kDa heparin-binding secreted growth factor that induces cell migration through binding to both its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) and integrin $\alpha_v\beta_3$. In the present work, we studied the effect of PTN on the generation of reactive oxygen species (ROS) and the involvement of ROS in PTN-induced cell migration.

METHODS

Human umbilical vein endothelial, human glioma M059K, Chinese hamster ovary and human prostate cancer LNCaP and PC3 cells were used. Intracellular ROS production was assayed using 5(6)-carboxy-2,7-dichlorodihydrofluorescein diacetate. Migration assays were performed in 24-well microchemotaxis chambers. RPTPβ/ζ was suppressed using small interfering RNA. The significance of variability between the results from each group and the corresponding control was determined by unpaired t-test.

RESULTS

PTN significantly increased endogenous ROS levels in a concentration and time-dependent manner, while in cells with down-regulated endogenous PTN expression, ROS levels were significantly decreased. Suppression of RPTPβ/ζ or $\alpha_v\beta_3$ integrin using genetic and pharmacological inhibitors or inhibition of c-src kinase activity abolished PTN-induced ROS generation. Phosphorylation of β_3 Tyr773 and phosphoinositide 3-kinase (PI3K) or Erk1/2 activation seem to be required, similarly to what has been previously shown for PTN-induced cell migration. Finally, ROS scavenging and xanthine oxidase inhibition completely abolished both PTN-induced ROS generation and cell migration, while NADPH oxidase inhibition had no effect.

CONCLUSIONS

These data suggest that xanthine oxidase-mediated ROS production is required for PTN-induced cell migration through the cell membrane functional complex of $\alpha_v\beta_3$ and RPTPβ/ζ and activation of c-src, PI3-K and ERK1/2 kinases.

Acknowledgement: This work was supported by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: "Heracleitus II. Investing in knowledge society through the European Social Fund".

PP-46

DEPRESSION, ANTIDEPRESSANT TREATMENT AND SURROGATE MARKERS OF ATHEROSCLEROSIS

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BACKGROUND AND OBJECTIVES

Major Depression is twice more common in women and sex differences exist in antidepressant pharmacokinetics and pharmacodynamics. Additionally, depression is recognized as a risk factor for cardiovascular disease. In this study we aim to investigate whether treatment of male and female depressed patients would impact on surrogate markers of atherosclerosis.

METHODS

In this study, depressed patients treated by their physician with citalopram 20-60mg and risperidone 0.5-1mg were invited to be examined at baseline and 21 weeks after treatment initiation. Surrogate markers of atherosclerosis were assessed using a validated non-invasive protocol.

RESULTS

In this on-going-study 35 patients completed their follow-up vascular assessments after 21-24 weeks of treatment. Those patients received a mean citalopram dose of 37 mg and a mean risperidone dose of 0.72 mg. A remarkable 70% of female patients responded to treatment in comparison to only 45% of male patients. No significant differences were found in blood biochemistry, except prolactin, which was increased on average by 50%. Interestingly, arterial stiffness, a surrogate marker of atherosclerosis, was significantly decreased following treatment ($p<0.001$, repeated measures analysis of variance).

CONCLUSIONS

Our preliminary results show that successful pharmacological treatment of major depression also results in an improvement of arterial stiffness, a surrogate marker of atherosclerosis. Additionally, a significant sex-difference was observed in response to the combined antidepressant/antipsychotic treatment of major depression.

PP-47

SEX DIFFERENCES IN RESPONSE TO ENVIRONMENTAL ENRICHMENT: BEHAVIORAL, NEUROCHEMICAL AND Brain Plasticity FINDINGS

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BACKGROUND AND OBJECTIVES

Early life experiences exert long-lasting effects on neuronal structure, function and connectivity and thus they play an important role in brain development, behaviour, as well as in the appearance of neuropsychiatric disorders. Marked sex differences are present in several neuropsychiatric disorders including depression, Alzheimer's and neurodevelopmental diseases. Environmental enrichment (EE) has been shown to have beneficial effects on brain plasticity and has been proposed as a therapeutic intervention. In the present study, we investigated sex differences in the effects of EE on cognitive, neurochemical and neuronal structure indices related to neuropsychiatric disorders.

METHODS

Male and female Wistar rats were reared in a standard or an enriched environment from P0-P90. Behavioural response was assessed in the open field and the novel object recognition test. Subsequently, rats were sacrificed and the serotonergic, dopaminergic and glutamatergic neurotransmission was evaluated in the hippocampus. Finally, protein markers indicative of neuronal plasticity were assessed in the same brain region with Western blot analysis.

RESULTS

In the novel object recognition test both EE males and females had a higher preference index for the novel object. Corticosterone serum levels were reduced only in males exposed to EE. Females exposed to EE had low glutamate levels and high serotonergic activity in their hippocampus. Western blot analysis revealed alterations in cytoskeletal and synaptic proteins, such as enhanced hippocampal PSD-95 in EE females.

CONCLUSIONS

Present findings reveal the potential significance of male or female sex in supporting the beneficial effects of environmental stimuli, which include enhancement of brain plasticity and cognition.

PP-48

PREFRONTAL CORTICAL – HIPPOCAMPAL COMMUNICATION AND ANTIDEPRESSANT TREATMENT IN RATS: EXPLORING SEX DIFFERENCES

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BACKGROUND & OBJECTIVES

Depression is twice as much prevalent in women, with sex differences in the onset, symptomatology and response to treatment. However, specific neurobiological underpinnings for this differentiation are still under rigorous research. Robust results from our laboratory indicate that disruption of the prefrontal cortical (PFC) – hippocampal network circuit via lesion of their interconnecting node, nucleus reuniens (RE), prevents the appearance of depressive-like behavior in male rats. In the present study, we sought to investigate whether the antidepressant-mimicking effect of the PFC – hippocampal circuit disruption would be replicated in female rats.

METHODS

Adult male and female rats received an excitotoxic RE lesion, or a sham-operation. After one week of recovery, rats were tested in the forced swim test (FST). Between the two FST sessions, rats received a standard subacute treatment consisting of 3 injections of sertraline (an SSRI, 10 mg/kg) or clomipramine (a tricyclic antidepressant, 10 mg/kg) or vehicle.

RESULTS

Male and female RE-lesioned rats had lower immobility and enhanced swimming duration than sham controls in the FST. Sertraline and clomipramine treatment reduced immobility duration in sham-operated rats, as expected. SSRI treatment in RE-lesioned rats reduced immobility only in females.

CONCLUSIONS

Our results uncover that the communication of PFC and hippocampus, depending on RE, is required for the appearance of depressive-like behavior in both sexes. Additionally, following disruption of the PFC-hippocampus circuit, antidepressant treatment elicits a sexually dimorphic behavioral profile.

PP-49

SUBACUTE AROMATASE INHIBITION EXHIBITS ANTIDEPRESSANT POTENTIAL IN FEMALE RATS

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BACKGROUND AND OBJECTIVES

Aromatase inhibitors, such as letrozole, block the production of estrogens and are used for the treatment of hormone-responsive breast cancer in women. In the present study we investigated whether decreased estrogen synthesis following letrozole treatment leads to a depressive-like behavioral response in female rats.

METHODS

Adult cycling female Sprague-Dawley rats were subjected to the forced swim test (FST), which is a test for antidepressant potential. Females were treated with either vehicle or the antidepressant fluoxetine for 28 days, in combination with subacute letrozole (3 injections in 24 hours) or sustained (7 days, 1 injection/day) letrozole treatment. Behavioral response in the FST was evaluated with the use of the software *Kinoscope*. Also, gonadal hormone levels were assayed following behavioral testing.

RESULTS

Immobility duration in the FST was reduced following acute aromatase inhibition, indicating letrozole's antidepressant potential. Instead, aromatase inhibition for one week did not show an antidepressant response. Estrogens levels were undetected following letrozole treatment, as expected. Moreover, serum testosterone levels were elevated following acute letrozole treatment and this was associated with the decreased depressive behavior in the FST. On the other hand, progesterone levels explained the increased swimming behavior in the FST.

CONCLUSIONS

These findings indicate that aromatase inhibitors may have psychotropic attributes that depend on treatment duration. Further preclinical and clinical studies are needed to verify the value of these findings and evaluate whether there is a role for aromatase inhibitors in antidepressant response.

PP-50

CANNABINOID ANTAGONISM BLOCKS THE EXPRESSION OF COCAINE CONDITIONED PLACE PREFERENCE

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BACKGROUND AND OBJECTIVES

The potential of endocannabinoid modulation for the treatment of psychostimulant addiction has been recently suggested. Here we studied the modulatory role of the endocannabinoid system on the rewarding effects of cocaine, using the conditioned place preference paradigm in the rat. We tested the potential of cannabinoid CB1 and CB2 agonists and antagonists (i) to affect the acquisition of cocaine conditioned place preference (Coc CPP), by administering cannabinoid compounds during cocaine Conditioning and (ii) to affect the expression of CocCPP, by administering the cannabinoid compounds on Test Day.

METHODS

Coc (20mg/kg) CPP was performed with an unbiased design in a three-compartment apparatus, with 1 habituation day (15 min free exploration), 8 days of conditioning with alternating cocaine and saline injections (30 min in black or white chamber), and 1 test day (15 min free exploration).

RESULTS

The results showed that the cannabinoid inverse agonist/antagonist SR 141716A decreased the acquisition (at 3mg/kg) and blocked the expression (at 1 and 3 mg/kg) of cocaine CPP. These effects were not due to direct effects of SR141716A on the conditioning procedure since SR141716A alone did not produce place reference.

CONCLUSIONS

Our findings show that the endocannabinoid system contributes to the primary rewarding properties of cocaine and, more so, to the expression of cocaine x environment associations. These results suggest that pharmacological intervention of the endocannabinoid system may be of value in the weakening of context-induced cocaine preference.

PP-51

LOW DOSES OF ENDOCANNABNOID AGONISTS DIRSUPT OBJECTRECOGNITION VIA CANNABINOID TYPE 1 RECEPTORS

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BACKGROUND AND OBJECTIVES

The therapeutic potential as well as the widespread recreational use of cannabis prompted us to study whether cannabinoids affect cognitive functions in the rat. We studied the effects of cannabinoid agonists on novel object recognition (NOR) because (i) this behavioral test is based on a spontaneous rodent exploratory behavior and (ii) there were limited and conflicting findings in the literature. One reason behind the inconclusive NOR studies was the use of high, motor-suppressant doses, which interfered with the exploratory behavior of the animals.

METHODS

Therefore, we determined the effects of endocannabinoid agonists on NOR, using a range of relatively low doses that did not affect vertical exploratorybehavior (HU 210, 0.001-0.03; WIN 55212-2, 0.1 – 1mg/kg) or produced a modest decrease in ambulation (WIN 55212-2, 1 mg/kg).We assessed the effects of cannabinoids on (i) short term memory (intertrial interval 1 h), (ii) long-term memory (intertrial interval 24 hrs), and (iii) long-term information retrieval (intertrial interval 24 hrs).

RESULTS

Cannabinoid agonists (HU210, 0.003 mg/kg and WIN 55212-2, 0.3mg/kg) impaired short-term NOR memory, without affecting total object exploration time. In addition, WIN 55212-2 (0.3mg/kg) also impaired long-term NOR memory and long-term NOR retrieval (without affecting total object exploration time) and these disruptions were prevented if a low dose of SR141716A (0.03 mg/kg) was administered before WIN55212-2.

CONCLUSIONS

These results show that activation of CB1 receptors affects all components of recognition memory including consolidation, storage and retrieval and further support the deleterious role of CB1 activation in cognitive function.

PP-52

THE ROLE OF ALPHA-ADRENERGIC BLOCKERS IN THE TREATMENT OF CHRONIC PROSTATITIS/CHRONIC PELVIC PAIN SYNDROME

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BACKROUND & OBJECTIVES

Chronic prostatitis/chronic pelvic pain syndrome is a debilitating disease of the male population, without a confirmed cause and without a curative treatment. Lower urinary tract symptoms, pelvic pain and sexual dysfunction are predominant in this entity. All these symptoms in combination with the absence of drug treatment results in a negative impact on the patients' quality of life. The purpose of this review is to present the role of alpha-adrenergic blockers in the treatment of chronic prostatitis/chronic pelvic pain syndrome, their mechanism of action and also the results of major clinical trials where these drugs have been used.

METHODS

A review of the literature in electronic databases Medline, Scopus and Cochrane Library was conducted. Additionally, websites of pharmaceutical companies, international urological guidelines and the most relevant international conferences were researched.

RESULTS

Out of the 51 retrieved articles, 7 were included in the present review. In most of these trials, the symptoms of patients were suppressed with the administration of a-blocker. In these patients, drug prescription is recommended for a specific long period of time in order to achieve the optimal result. On the contrary, two studies did not find statistically significant difference in the improvement of symptoms by the administration of a-blockers compared to placebo.

CONCLUSIONS

Clinical studies including a larger number of patients need to be conducted in order to find the most appropriate drug treatment. The positive indications that exist, concerning the good response of patients towards this treatment in combination with its low cost, further support this need for additional research in this field.

PP-53

INFLUENCE OF CHRONIC AND SUB-CHRONIC SOY DIET ON THE REPRODUCTIVE SYSTEM OF ADULT MALE RATS

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BACKGROUND AND OBJECTIVES

Soy diet may be beneficial during menopause and old age but may have adverse effects on reproduction, when consumed during reproductive age. The purpose of this study was to investigate the effect of chronic and sub-chronic soy diet on the reproductive system of adult male rats.

METHODS

Four groups of adult male albino Wistar rats, 6 months old, were used: two study and two control groups. The study groups received ad libidum food enriched in soy protein for 6 weeks (group 1) and 12 weeks (group 2). The control groups received ad libidum the standard food for 6 weeks and 12 weeks (groups 3 an4 respectively). All groups were grown under the same conditions and according to the rules of GLP. The study was approved by the local Ethics Committee. After sacrifice, specimens of the reproductive organs were prepared and stained for electron microscopic examination. The preparations were observed by two different persons, blinded to the source of the specimen. Statistical analysis was performed with the statistical package SPSS.

RESULTS

Ultrastructural changes of the seminiferous epithelium were observed in the study groups; many spermatocytes appeared with endocytoplasmic and perinuclear oedema. The number of spermatozoa in the reproductive tract was much lower in group 2 comparatively to group 4; in group 1 it was similar to the number of spermatozoa in group 3.

CONCLUSIONS

Chronic and sub-chronic soy diet caused ultrastructural changes in the seminiferous epithelium of adult rats; only chronic soy diet caused adverse effects on spermatogenesis.

PP-54

ATTITUDES IN ANTIDEPRESSANTS USE IN THESSALONIKI

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BACKGROUND AND OBJECTIVES

The use of generics in the Greek market of medicines is generally lower than in other European countries. The purpose of this work was to study the attitudes in antidepressants use, and to calculate the use of generics in antidepressant sales in a sample from the medicines market of Thessaloniki.

METHODS

A sample of antidepressants registered sales was collected from the new registration system, which has been applied during the last two years in Greece. The sample corresponded to a small amount of sales from the market of Thessaloniki during the years 2012 and 2013. All classes of antidepressants and their relative ratios in the sales were estimated, and the percentage of generics in the sale of each medicine was calculated out of a variety of brand names in each class of antidepressants. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics.

RESULTS

Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) corresponded to 88% of total antidepressants sales (42,000 out of 47,644 DDDs). Generic use corresponded to 28% of total sales and varied greatly, being 14% of fluoxetine sale (1,066 out of 7,834 DDDs), 35% of venlafaxine sale (593 out of 1685 DDDs), 36% of sertraline sale (2,972 out of 8,294 DDDs) and 53% of citalopram sale (5,708 out of 10,696 DDDs).

CONCLUSIONS

The percentage of generics in antidepressants use was low in the studied sample, being higher than 50% only for citalopram.

PP-55

SERUM HISTAMINE LEVELS IN ALLERGIC BEEKEEPERS AS POTENTIAL FACTOR TO DETERMINE THERAPEUTIC STRATEGIES

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BACKGROUND & OBJECTIVES

Beekeepers, being heavily exposed to honeybee stings, are at increased risk of becoming allergic, while many outgrow their allergy during beekeeping. The underlying mechanisms remain elusive, and biomarkers that would distinguish between tolerant and non-tolerant beekeepers are not currently available. This preliminary study sought to investigate the value of serum histamine (*s*HI) level determination in tolerant and non-tolerant allergic beekeepers as a marker of prognostic and therapeutic relevance.

METHODS

Eleven medication-free allergic beekeepers were selected, out of 472 volunteers, for the determination of *s*HI levels, based on a series of exclusion criteria, including allergy history. Six individuals had the last bee sting experience >200 days (past BS) and 5 during 30 days (recent BS) before blood sampling. *s*HI levels were quantified fluorophotometrically and evaluated by appropriate non parametric statistical analyses.

RESULTS

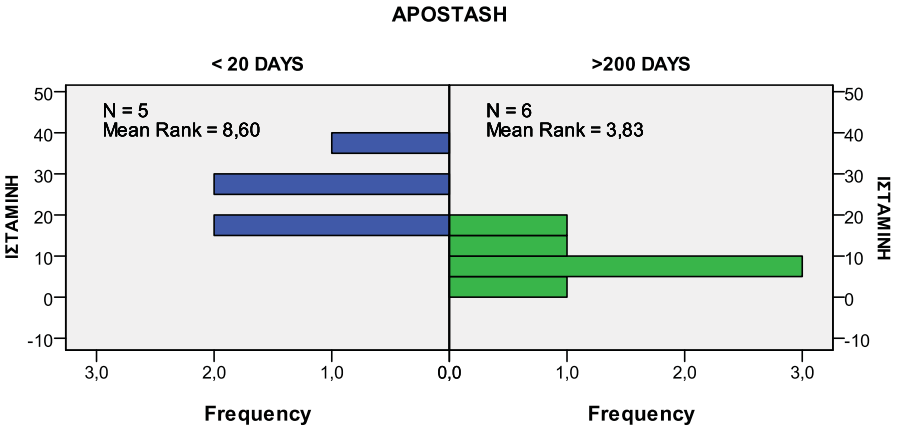
*s*HI levels in the beekeepers were not statistically different from those determined in 5 non-beekeeping matched volunteers. The level of *s*HI was higher in the blood of tolerant compared to non-tolerant beekeepers, the distribution being wider in the non-tolerant group. Similarly, *s*HI levels were higher in the recent compared to the past BS group, while no significant difference was observed between the tolerant and the non-tolerant past BS subgroups.

CONCLUSIONS

Despite the small sample size, the findings provide first indication for further examining the potential exploitation of *s*HI quantification as a prognostic factor that would facilitate the determination of optimal treatment strategies in allergic beekeepers.

This work was partly supported by the UoA grant 70/4/5900.

Independent-Samples Mann-Whitney U Test



Total N	11
Mann-Whitney U	28,000
Wilcoxon W	43,000
Test Statistic	28,000
Standard Error	5,465
Standardized Test Statistic	2,379
Asymptotic Sig. (2-sided test)	,017
Exact Sig. (2-sided test)	,017

PP-56

RANITIDINE-INDUCED ANAPHYLAXIS: A CASE REPORT

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BACKGROUND & OBJECTIVES

Drug induced anaphylaxis is a constantly increasing medical emergency. We present a case of a severe anaphylaxis to ranitidine, a commonly used H_2 receptor inverse agonist (H_2R*ia*) and the relevant diagnostic procedure supplemented by blood histamine measurements.

METHODS

A young girl was referred to our unit shortly after a severe anaphylactic reaction during preoperative anesthesia. All drugs used preoperatively were tested through skin prick and intradermal application. Among them, only ranitidine produced positive reactions and was thus considered the culprit drug. In order to determine possible cross-reactivity between agents of the same class, other H_2R*ia* were tested, including cimetidine and famotidine, with no positive reactions whatsoever. Graded challenges -the gold standard of drug allergy diagnosis- to intravenous ranitidine and cimetidine (as a possible therapeutic alternative) were conducted. Histamine levels in both serum and whole peripheral blood were determined fluorometrically before, 30' after the initiation of any reaction and at the end of an uneventful challenge.

RESULTS

The patient's basal serum histamine levels were comparable to those of normal subjects, whereas basal whole blood histamine levels were higher. During challenge to ranitidine the patient developed allergic symptoms, including rash, headache and tachycardia, and the procedure was interrupted. Serum histamine levels were increased from 4.3ng/mL to 17.1ng/mL, while they remained low after the negative provocation to cimetidine.

CONCLUSIONS

Serum histamine measurement is a useful tool to document anaphylactic reactions especially in the context of a controlled challenge. In accordance to previous reports, no cross-reactivity between H_2R*ia* was observed.

PP-57

EXPLORING THE CHARACTERISTICS OF DRUG ALLERGY IN THE GREEK POPULATION

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BACKGROUND & OBJECTIVES

Since there are no epidemiologic data on the incidence of drug hypersensitivity reactions (DHR) in the Greek population, we conducted a descriptive, observational study in order to document and classify the cases of drug allergy in the outpatient clinic settings of a tertiary care hospital.

METHODS

Patients visiting the Drug Allergy Outpatient Clinic (OC) of our Unit were enrolled. Data was retrieved from OC patients only, Emergency Room patients and referrals from other clinics being excluded.

RESULTS

1129 patients (304 male, 824 females, mean age 45.9±17,9 years) were evaluated. 401 patients (35.6%) experienced an immediate reaction, while 575 patients (50.9%) reported a non-immediate reaction. Almost one third of the patients (340 pts, 30.1%) reacted to a single drug (single-reactors), while 519 patients (46%) reported reactions to more than one drug (multi-reactors). The principal cause of DHR was β-lactams (45.5%), followed by NSAIDs (13%) and macrolides (6.8%). Within the β-lactam reactors group, 304 (60.6%) and 196 (39.1%) patients reported a reaction to a synthetic penicillin and to cephalosporins, respectively. Amoxicillin was involved in the 24% of all DHRs reviewed and was by far the most frequently involved drug.

CONCLUSIONS

Most of the obtained results are in accordance with the existing literature: female predominance in drug allergy is universal, and β-lactams and NSAIDs are typically the "usual suspects" behind DHRs. However, the high rate of allergy to cephalosporins could be considered as characteristic for the Greek population and possibly correlate to the local physicians' prescription habits.

PP-58

NOVEL MULTI – TARGETED COMPOUNDS WITH ACTIVITY IN A HYPERLIPIDAEMIC/ DIABETES II MOUSE MODEL

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BACKGROUND AND OBJECTIVES

A correlation exists between oxidative stress, inflammation, hyperlipidaemia and the development of atherosclerosis/diabetes II. Simultaneously addressing these targets is considered beneficial in the treatment of such multifactorial diseases. The aim of this study is to evaluate the in vivo activity of two potent (in vitro) derivatives designed as multi – targeted agents against atherosclerosis/ diabetes II.

METHODS

SKH–2 male mice (7 – 8 weeks old) were divided into several groups, receiving either a standard chow diet or a high fat diet (40% w/w fat) ± multiple low–dose of streptozotocin, with or without the administration of compounds **1**, **2** or reference compounds (at a dose of 56 μmol/Kg/twice daily for 50 days). At predetermined time points, glucose levels, lipid parameters, body weight and food consumption were estimated.

RESULTS

The average body weight and food consumption exhibited no significant differences among groups over time and throughout the experiment. After 50 days of treatment, glucose levels of mice treated with compound **1** or **2** were decreased by 38% ($^{***}P<0.0001$) and 41% ($^{***}P<0.0001$) compared to control levels. Total cholesterol levels decreased by 51% ($^{***}P<0.0001$) and 31% ($^{***}P=0.0007$), triglyceride levels by 83% ($^{***}P<0.0001$) and 78% ($^{***}P<0.0001$), LDL–cholesterol levels by 52% ($^{**}P = 0.005$) and 46% ($^{***}P=0.0002$), while the antiatherogenic index (HDL/LDL ratio) improved by 40% ($^{*}P = 0.033$) and 28% ($^{**}P=0.001$), respectively. Both compounds exhibited more potent activity, as shown by almost all parameters measured, compared to reference compounds that were also hereby evaluated.

CONCLUSIONS

Results of these in vivo experiments confirm the increased activity of the designed multi–targeted derivatives **1** and **2** compared to reference compounds.

References

Lipoic acid: energy production, antioxidant activity and health effects. Ed. M. S. Patel and L. Packer, CRC Press, **2008**. *New Multifunctional Di-tert butyl-phenoloctahydro-(pyrido/benz)oxazine Derivatives with Antioxidant, Antihyperlipidemic, and Antidiabetic Action*. Ladopoulou E, Matralis A, Kourounakis A. *J Med Chem.* **2013**, 56(8):3330. *Multi-targeted drug design approaches for multifactorial diseases: from neurodegenerative to cardiovascular applications.* M. Katselou, A. Matralis and A. Kourounakis. *Curr. Med. Chem.* **2014.** *Design of Novel Potent Antihyperlipidemic Agents with Antioxidant/Anti-inflammatory Properties; Exploiting Phenothiazine's Strong Antioxidant Activity*. A. Matralis and A. Kourounakis *J Med Chem,* **2014.**

PP-62

TARGETING DNA METHYLATION BY GREEN TEA CATECHINS

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BACKGROUND & OBJECTIVES

Recent data have shown strong chemopreventive and possibly cancer chemotherapeutic effects of green tea catechins against cancer. Although the molecular mechanisms of the anti-proliferative action of green tea catechins have not been delineated, green tea catechins seem to be multi-target agents, modulating multiple signalling pathways. In addition green tea catechins have been reported to modulate epigenetic processes. Aberrant epigenetic alterations in the genome such as DNA methylation play a significant role in cancer development. Indeed, epigenetic processes have been recognized as a new target for anticancer drug design. This review aims to synthesize evidence on the modulation of DNA methylation by green tea catechins.

METHODS

Electronic databases were searched with the appropriate search terms up to and including February 2013.

RESULTS

Green tea catechins have been reported to reverse DNA methylation of tumor suppressor genes and increase transcription of these genes. Green tea catechins and especially EGCG modulate DNA methylation by attenuating the effect of DNMT1. However, the exact mechanism of DNMT1 inhibition is not delineated. Suggested mechanisms include direct enzymatic inhibition, indirect enzymatic inhibition, reduced DNMT1 expression and translation. The possible effect of green tea catechins on other pathways of DNA methylation i.e. methyl-CpG binding domain (MBD) proteins has not been investigated. Furthermore, the link between redox properties and epigenetic modulation by green tea catechins has also not been defined.

CONCLUSION

Since green tea catechins are natural compounds with a rather acceptable safety profile, further research on their action as inhibitors of DNA methylation seems worthwhile.

PP-63

EFFECT OF OPIOIDS ON INTERNEURONS OF THE DORSAL AND VENTRAL RAT HIPPOCAMPUS

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BACKGROUND & OBJECTIVES

The hippocampus is differentiated along its longitudinal axis in terms of the electrophysiological properties of its principal neurons, the anatomical characteristics of neuronal network and the differential role of the neurotransmitters and neuromodulators, including the opioids. The aim of the present study was to investigate the role of the Ih current of CA1 interneurons in the functional differentiation of the dorsal and the ventral hippocampus. The effect of morphine on Ih was also examined.

METHODS

Standard intracellular current clamp recordings were obtained *in vitro* from CA1 pyramidal neurons and interneurons of the oriens/alveus border in 500μm-thick transverse slices from dorsal and ventral rat hippocampus.

RESULTS

Interneurons of the dorsal and ventral hippocampus exhibited similar passive membrane properties. Conversely, interneurons of the ventral hippocampus showed a stronger Ih compared to interneurons of the dorsal hippocampus; moreover, Ih kinetics and the characteristics of the rebound depolarization following Ih activation were distinct between the two populations. Morphine (30 μM) hyperpolarized dorsal and ventral interneurons, reduced Ih current activation and changed the characteristics of the rebound depolarization; interestingly, in one interneuron the drug exhibited opposite results (i.e. caused membrane depolarization and Ih augmentation. Moreover, morphine decreased the frequency of spontaneous interneuronal firing at resting membrane potential and the firing frequency elicited in interneurons following sustained depolarizing pulses.

CONCLUSIONS

Interneurons of the dorsal and ventral hippocampus exhibit different intrinsic characteristics. Opioids reduce interneuron excitability in both dorsal and ventral hippocampus. Our findings confirm the complexity of the modulatory role of the opioids in the hippocampal network.

Acknowledgements: This work was supported by the Ministry of Education (Herakleitos I, Pythagoras II).

PP-64

HPLC DETERMINATION OF THE OXCARBAZEPINE ACTIVE METABOLITE LICARBAZEPINE IN HUMAN SERUM

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BACKGROUND & OBJECTIVES

Oxcarbazepine, a keto-derivative of carbamazepine indicated for the monotherapy or adjunctive treatment of partial seizures, is metabolized to the pharmacologically active metabolite 10-hydroxy-carbazepine, Licarbazepine. In the present study a simple, sensitive and reliable HPLC method was developed for the determination of Licarbazepine in the serum of patients undergoing therapy with Oxcarbazepine.

METHODS

250 μL of the Internal Standard (IS) (acetanilide 20μg/mL in methanol) was added in 100 μL serum or spiked samples. The samples were vortexed (15"-30"), left to stand (5') and centrifuged (13000rpm, 15', 4°C). The supernatant (20μL) was injected in the chromatographic column (Kromasil; C18; 250x4.6mm ID; 5μm particle size). The mobile phase consisted of 50mM phosphate buffer (pH 4) and acetonitrile 70/30 (v/v), delivered at 30°C, at a flow rate 0.8 mL/min. Licarbazepine and the IS were monitored at 210nm.

RESULTS

Licarbazepine and the IS eluted at 7.3±0.04 and 8.2±0.06 min, respectively. The calibration curve (peak areas of Licarbazepine versus IS) was linear at a range 0.5-50μg/mL. Analytical variation was 6%. Specificity was examined in blank serum samples and BIORAD TDM Quality Control containing 21 antiepileptic and commonly prescribed drugs. No interferences at the elution times of Licarbazepine or the IS were observed. Moreover, no interferences were observed at the elution times of 12 medications commonly administered in psychiatric patients (benzodiazepines, antidepressants, antipsychotics).

CONCLUSIONS

A simple, sensitive, reliable and low cost HPLC method was developed for the determination of Licarbazepine in human samples. The method can be implemented in the Therapeutic Drug Monitoring of Licarbazepine in epileptic patients and patients with bipolar disorders.

Acknowledgements: This work was supported by the Ministry of Health (KESY).

PP-65

PHOSPHOLAMBAN IS A FINAL EFFECTOR OF ISCHEMIC POSTCONDITIONING AND H_2S INDUCED CARDIOPROTECTION.

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BACKGROUND & OBJECTIVES

The novel gasotransmitter hydrogen sulfide (H_2S) has been shown to inhibit phosphodiesterase activity, increase cGMP levels and activate cGMP-dependent protein kinase (PKG), leading to vasodilation and angiogenesis. Protection by postconditioning (PostC) in the heart involves the activation of the cGMP/PKG pathway that, in turn, reduces apoptosis and inhibits mitochondrial permeability transition pores (mPTP), caused by increased intracellular Ca^{2+}. Preliminary data from our group showed that exogenous administration of H_2S induces pharmacological postconditioning through the activation of the PKG. As phospholamban (PLN) regulates cytosolic Ca^{2+} levels, we sought to determine the role of PLN in ischemic PostC and in H_2S-induced pharmacological PostC.

METHODS

Twenty three wild type Sv129J and twenty one PLN(-/-) homozygous knockout mice of both sexes were subjected to 30 minutes regional ischemia (isc) of the heart followed by 2 hours of reperfusion (rep). Wild type and PLN(-/-) animals were divided in three subgroups with the following additional interventions: 1) Control, no further intervention, 2) NaHS group, administration of NaHS as a bolus dose of $100\mu g.kg^{-1}$ at the 20th minute of sustained isc and 3) PostC group, application of 3 cycles of 10 seconds isc - 10 seconds rep immediately after prolonged isc. The infarct size (I) and the area at risk (R) were estimated in percent (%).

RESULTS

No significant differences were detected in the (R) among the studied groups. Control wild type had similar %I/R compared to PLN (-/-) control animals ($42.1 \pm 2.6\%$ vs $38.0 \pm 2.9\%$, p=NS). In wild type animals, PostC application and NaHS administration conferred a significant benefit compared to control ($14.9 \pm 2.1\%$ and $15.5 \pm 1.2\%$ respectively vs $42.1 \pm 2.6\%$, $p<0.05$). However, neither PostC nor NaHS conferred cardioprotection in PLN (-/-) animals ($45.2 \pm 2.1\%$ and $42.2 \pm 1.5\%$ respectively, $p<0.05$ vs wild type PostC and NaHS groups).

CONCLUSIONS

Our findings suggest that ischemic and pharmacological postconditioning induced by H_2S administration requires PLN activation.

PP-66

NITROGLYCERIN INDUCES PHARMACOLOGICAL POSTCONDITIONING THROUGH eNOS ACTIVATION INDEPENDENTLY OF PKG AND nNOS IN ANESTHETIZED RABBITS.

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BACKGROUND & OBJECTIVES

Nitric oxide (NO) is a key mediator of cardioprotection. However the effect of administration of exogenous NO donors at the end of ischemia (isc) as a pharmacological post-conditioning agent has not been studied in detail. We have previously shown that nitroglycerin (NTG) administered at the end of isc and during reperfusion (rep) reduces myocardial infarction and peroxynitrite formation. The purpose of the present study was to evaluate the contribution of the endogenous NO pathway to the cardioprotective action of NTG.

METHODS

Anesthetized male rabbits were subjected to 30-min regional myocardial isc and 3-hour rep and randomized into 13 groups as follows: 1) control, no further intervention, 2) postconditioning (PostC) with 8 cycles of 30-sec isc/rep at the onset of rep, 3) NTG treated (2 μg/kg-1/min-1) for a total time of 65 min starting 10 min prior to rep 4) NTG plus the NOS inhibitor L-NAME (iv bolus 10 mg/kg) at the 19th min of isc, 5) NTG plus the selective nNOS inhibitor 7-Nitroindazole (7-NI, iv bolus 50mg/kg) 10 min before isc, 6) NTG plus the PI3K inhibitor Wortmannin (iv bolus 60μg/kg) at the 19th min of isc, 7) NTG plus the adenosine receptor inhibitor SPT (iv bolus 10mg/kg) 20 min before isc, and 8) NTG plus the PKG inhibitor DT-2 (iv bolus 0,25mg/kg) 10 min before isc. In five additional groups, L-NAME, 7-NI, Wortmannin, SPT and DT-2 were administered alone at the same time and dose as previously described. After the end of the experiments the infarct size (I) and the area at risk (R) were estimated as % I/R. In a second series of experiments animals were subjected to the above interventions up to 10th min of rep when tissue samples were collected for measurement of eNOS and Akt levels and phospohrylation.

RESULTS

Co-administration of L-Name or Wortmannin along with NTG reduced the infarct limiting effects of NTG (37.9±2.0%, and 38.3±2.6% respectively vs 23.0±3.2%, $p<0.05$). Inhibition of adenosine receptors activation and PKG did not affect the cardioprotection afforded by NTG (16.0±1.8% and 18.5±3.4% respectively, p=NS vs NTG). Inhibition of nNOS increased NTG benefit (12.3±1.0%, $p<0.05$) whilst 7-NI itself exerted cardioprotection versus the control group (27.05±1.6% and 48.05±2.0%, $p<0.05$). eNOS and AKT phosphorylation was higher in NTG-treated animals compared to control ($p<0.05$).

CONCLUSIONS

Exogenous administration of NTG induces pharmacological postconditioning through activation of the PI3K-AKT pathway leading to further NO formation due to NOS activation; this effect is independent of adenosine receptors, PKG and nNOS and most likely mediated by eNOS.

PP-67

NOVEL ANTI-ARRHYTHMIC DRUGS: EVALUATION OF A KNOWN CAMKII INHIBITOR (KN-93) IN A MOUSE MODEL OF VENTRICULAR ARRHYTHMIAS

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BACKGROUND & OBJECTIVES

Junctin is a sarcoplasmic reticulum protein, which is down-regulated in patients with heart failure, while its ablation in mice is associated with stress-induced arrhythmias, due to increased Ca^{2+} leak. Interestingly, CaMKII mediated phosphorylation of RyR contributes to arrhythmogenesis by promoting Ca^{2+} leak. Therefore, CaMKII inhibition immerges as a promising therapeutic strategy for arrhythmias in the junctin knock out (JKO) mouse model. The purpose of this study was to elucidate the anti-arrhythmic effect of a CaMKII inhibitor (KN-93) in the JKO mouse model.

METHODS

The CaMKII inhibitor KN-93 was evaluated using biochemical, cellular (in vitro) and in vivo assays in the JKO model.

RESULTS

In vivo, KN-93 acute administration significantly reduced (65%) the occurrence of stress-induced (caffeine/epinephrine) malignant arrhythmias, such as ventricular tachycardia, in the JKO mice, as shown by ECG. In vitro, pretreatment of isolated cardiomyocytes with KN-93 significantly reduced the stress-induced aftercontractions (37%) and Ca^{2+} aftertransients (34%) under stress conditions. These effects are likely mediated through modulation of the SR Ca^{2+} release and uptake units, namely RyR2 and SERCA/PLN, which presented with significantly reduced CaMKII phosphorylation.

CONCLUSIONS

CaMKII inhibition by KN-93 can significantly reduce stress-induced malignant arrhythmias, such as NSVT, SVT and BDVT, in the JKO mouse model. This is mediated by reducing triggered activity, as well as attenuating the diastolic SR Ca^{2+} leak from RyR and the SR Ca^{2+} uptake from the SERCA/PLN complex. Thus, CaMKII inhibition might be a novel target for stress-induced malignant - but not mild - ventricular arrhythmias.

PP-68

"RECONSTITUTED HDL TREATMENT INDUCES ALTERATIONS IN PRIMARY HUMAN AORTIC ENDOTHELIAL CELL (HAEC) MIGRATION AND APOPTOSIS PATHWAYS"

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BACKGROUND & OBJECTIVES

HDL and Apolipoprotein A-I (apoA-I) have atheroprotective properties, and affect the functions of ECs through unknown mechanisms. The aim of this study was to investigate the effect of reconstituted HDL containing human apoA-I, cholesterol and phospholipids (rHDL-apoA-I) on the molecular mechanisms that control EC migration, apoptosis and survival.

METHODS

Primary HAEC were treated with rHDL-apoA-I or PBS. Total RNA was isolated and analyzed by whole genome microarrays (Gene 1.0 ST Array/Affymetrix) followed by bioinformatical analysis (2-fold and ≤0.05 FDR thresholds) and high-throughput qRT-PCR validation of 47 genes. Data-mining involved cutting-edge software and extensive PubMed-based searches.

RESULTS

Significant changes in expression were observed in 188 genes. Sixteen of these genes were related to EC migration that are involved in the activation of the following pathways: 1) The small RHO GTPases-mediated migration pathway that leads to cytoskeletal rearrangement, 2) the PI3K/AKT pathway that induces EC migration through either generation of NO by eNOS (endothelial nitric oxide synthase) activation or MMP-2/9 (matrix metalloproteinase-2/9) activation which promotes cell migration through extracellular matrix degradation and 3) the ERK1/2 pathway that stimulates EC migration through cytoskeletal reorganization and activation of MMP-2/9. Confirmed changes were also observed in 10 EC apoptosis/survival-associated genes after rHDL-apoA-I treatment that are involved in: 1) P53-dependent apoptosis, 2) Cytochrome C-mediated apoptosis and 3) PI3K/AKT survival pathway.

CONCLUSIONS

rHDL-apoA-I affects EC migration mechanisms through cytoskeleton reorganization. rHDL-apoA-I also affects genes implicated in EC apoptosis/survival pathways. Through these changes rHDL-apoA-I could in principle preserve endothelial integrity and thus protect from atherosclerosis.

PP-69

"PHARMACOGENOMIC ASSESSMENT OF THE CNTF EFFECTS ON STRIATED MUSCLES»

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BACKGROUND

The Ciliary Neurotrophic Factor (CNTF) is a pluripotent cytokine with an anorexigenic effect in the hypothalamus, similar to leptin. Recent data demonstrate that, apart from inducing a feeling of satiety, CNTF improves insulin sensitivity, increases energy expenditure and induces greater weight loss compared to pair-fed controls.

METHODS

Skeletal muscles are implicated in metabolism and may contribute to the effect of CNTF. To address this question and identify the specific molecular mechanisms, we used a mouse model of diet-induced obesity, to which CNTF was administered for 7 days. Dissected soleus muscles were analyzed at the whole genome expression level using Affymetrix Mouse Gene 1.0ST microarrays.

RESULTS

Significant changes were observed in a number of genes, with the most significant and relevant molecular pathways including: muscle differentiation (e.g. Ankrd2, Mamstr, Csrp3, Fbxo32), cytoskeletal restructuring (e.g. Col3a1, Tubb6, Mylk2), regulation of Calcium circulation (e.g. Casr, Chrna1, S100a8) and regulation of metabolism (e.g. Pak1, Lep, Pfkfb3).

CONCLUSIONS

Overall the results reflect a process of myogenic growth, which may be related to the improvement of the broader metabolic profile and sensitization of the organism to insulin. These findings set the foundations for an understanding of the molecular effect of CNTF and the characterization of the role skeletal muscle can play in combating obesity and the metabolic syndrome.

Notes