ROYAL ACADEMY OF MEDICINE IN IRELAND

SECTION OF BIOMEDICAL SCIENCES

Annual Meeting 2013

Including Donegan Medal and
Barcroft Medal Competitions
&
Conway Review Lecture

20th June 2013

Brookfield Health Sciences Complex,
University College Cork

UCC
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

PROGRAMME & ABSTRACTS

Sponsors:

red box direct
Think inside the box

Roche

SARSTEDT
Your partner in medicine and science worldwide...

VWR

ION OPTIX

ADINSTRUMENTS

MSC

WPI
World Precision Instruments

ROYAL ACADEMY OF MEDICINE IN IRELAND

Section of Biomedical Sciences Annual Meeting
20th June 2013
Brookfield Health Sciences Complex, UCC

Section President: Professor Aidan Bradford, RCSI
Section Secretary: Professor Ken D. O'Halloran, UCC

ON-SITE REGISTRATION:	**08.30-09.10**
Welcome	09.10-09:15
Donegan Communications: D1-D7 Chair: Prof. Ken O'Halloran, UCC	09.15-11.00
Poster Session *Coffee/Tea will be served.*	11.00-11.45
Donegan Communications: D8-D11 Chair: Prof. Ken O'Halloran, UCC	11.45-12.45
Oral Communications: C1-C3 Chair: Dr. Michelle Roche, NUIG	12.45-13.30
LUNCH **Restaurant in Brookfield Health Sciences Complex** *(Council Meeting RAMI Section of Biomedical Science; poster viewing)*	**13.30-14.30**
Oral Communications: C4-C8 Chair: Dr. Eric Downer, UCC	14.30-15.45
Barcroft Communications: B1–B3 Chair: Prof. James Jones, UCD	15.45-16.45
Poster Session *Coffee/Tea will be served.*	16.45-17.15
Conway Review Lecture Professor John F. Cryan, UCC *A gut feeling about brain function*	17.15
Presentation of Prizes	18.15
Conference Dinner	19.30

REGISTRATION	**08.30-09.10**
OPENING ADDRESS	**09.10**

Donegan Medal	**09.15-11.00**
Communications D1-D7	

D1 09.15

INVESTIGATING THE EFFECTS AND PUTATIVE MECHANISM OF ACQUIRED RESISTANCE TO NERATINIB IN BREAST CANCER

S. Breslin, C. Corcoran, S. Rani, L. O' Driscoll.
School of Pharmacy and Pharmaceutical Sciences & Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland.

D2 09.30

CLOSING A GAP IN SKELETAL MUSCLE EXCITATION-CONTRACTION COUPLING: THE 90 kDa JUNCTIONAL FOOT PROTEIN IS JUNCTOPHILIN

K.K. English[1], A. Carla[2], V. Sorrentino[2], J.J. Mackrill[1].
[1] Department of Physiology, University College Cork, Ireland and
[2] Molecular Medicine Section, Department of Neuroscience and Interuniversity Institute of Myology, University of Siena, Siena, Italy.

D3 09.45

INVESTIGATING THE MOLECULAR MECHANISMS THAT UNDERPIN MOUSE RESPIRATORY MUSCLE ADAPTATION TO CHRONIC HYPOXIA: A REDOX PROTEOMICS APPROACH

P. Lewis[1], D. Sheehan[2], K.D. O'Halloran[1].
Departments of [1]Physiology and [2]Biochemistry, University College Cork, Ireland.

D4 10.00

^{13}C-GLUCOSE BREATH TEST IN THE DIAGNOSIS OF DIABETES IN HUMANS

L. Lynch, A. Shafat
Physiology, School of Medicine, National University of Ireland, Galway, Ireland.

D5 10.15

GASTROINTESTINAL CONTRACTILE ACTIVITY IN THE *MDX* MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY, A ROLE FOR IL-6 IN PATHOPHYSIOLOGY

J. Manning, D. O' Malley.
Department of Physiology, University College Cork, Cork, Ireland.

D6 10.30

OESTROGEN AND HYPOXIA MODULATE GENE EXPRESSION IN COLORECTAL CANCER CELLS THROUGH SPECIFIC MICRORNA REGULATION

Á. Nolan[1], I. Bray[2], R. Stallings[2], A. Silver[3], W. Thomas[1], B. Harvey[1].
Departments of [1]Molecular Medicine and [2]Cancer Genetics, Royal College of Surgeons in Ireland, Dublin, Ireland and [3]Colorectal Cancer Genetics, Barts and the London School of Medicine and Dentistry, London, UK.

D7 10.45

THE EFFECTS OF HYPOXIA-MIMETIC DRUGS ON SYNAPTIC TRANSMISSION AND SYNAPTIC PLASTICITY IN THE DENTATE GYRUS IN VITRO

H. Nolan, A.E. Corcoran, J.J. O'Connor.
UCD School of Biomolecular and Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

Poster Viewing 11.00-11.45
Authors to attend. Tea/Coffee will be served. Please visit the exhibitors.

Donegan Medal 11.45-12.45
Communications D8-D11

D8 11.45

ALPHA-1 ANTITRYPSIN AS A POTENTIAL THERAPY TARGETING LEUKOTRIENE B_4 MEDIATED RESPIRATORY INFLAMMATION

C.A. O' Dwyer, N. Banville, N.G. McElvaney, E.P. Reeves.
Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland.

D9 12.00

THE EXPRESSION OF NPY 1 RECEPTOR IN EXPERIMENTAL TEMPORAL LOBE EPILEPSY

E. O'Loughlin[1], K. McDermott[1] and D. Yilmazer-Hanke[1, 2].
[1] Department of Anatomy, University College Cork, Cork, Ireland and
[1,2] Department of Biomedical Sciences, Creighton University, Omaha, USA.

D10 12.15

DECREASED NEUTROPHIL MEMBRANE CHOLESTEROL CONTENT CAUSES LIPID RAFT STABILITY, IN PART EXPLAINING DYSREGULATED NEUTROPHIL ACTIVITY IN CYSTIC FIBROSIS

M. White, S. Cox, E. Hayes, N.G. McElvaney, E.P. Reeves.
Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland.

D11 12.30

EFFECT OF P-GLYCOPROTEIN INHIBITION ON THE BRAIN DISTRIBUTION AND ANTIDEPRESSANT-LIKE ACTIVITY OF ESCITALOPRAM IN RODENTS

F.E. O'Brien[1,2,3], R.M. O'Connor[3], G. Clarke[1,4], T.G. Dinan [1,4], B.T. Griffin[2], J.F. Cryan[1,3]
[1]Alimentary Pharmabiotic Centre, [2]School of Pharmacy, [3]Department of Anatomy & Neuroscience, and [4]Department of Psychiatry, University College Cork, Ireland.

Oral Communications 12.45-13.30
Communications C1-C3

C1 12.45

CORRECTION >80% OF CF CAUSING MUTATIONS IN CF CELLS USING ZFN HOMOLOGY-DIRECTED REPAIR

J.A. Hollywood[1,2], C.M. Lee[1,2] , K. Kaschig[1,2] , R. Flynn[1], M.F. Scallan[2], P.T. Harrison[1]
[1]Department of Physiology & BioSciences Institute and [2]Department of Microbiology, University College Cork, Cork, Ireland.

C2 13.00

APATHY & PARKINSON'S DISEASE: A HIGH ANGULAR RESOLUTION DIFFUSION IMAGING STUDY

W. Quinn[1,2], T. Vidal[3], I. Rieu[3], M. Perie[3] , F. Durif[2,3].
[1]Cork Institute of Technology, Cork, Ireland, [2]Université d'Auvergne, Clermont-Ferrand, France, and [3]Hôpital Gabriel Montpied, Clermont-Ferrand, France.

C3 13.15

SENSOR DEVICES FOR HEALTH APPLICATIONS BASED ON SMART MINIATURISED MICROSYSTEM TECHNOLOGY

E. Moore, W. Messina, U. Crowley, G. Duffy, M. Fitzgerald
Life Science Interface, Tyndall National Institute, University College Cork, Cork, Ireland.

LUNCH 13.30-14.30
Poster Viewing

COUNCIL MEETING 14.00-14.30
Biomedical Sciences Council Members only

Oral Communications 14.30-15.30
Communications C3-C6

C4 14.30

A NOVEL PLATELET FUNCTION TEST RAPIDLY DETECTS DRUG EFFECTS IN PATIENTS WITH CARDIOVASCULAR DISEASE

K. Egan[1,2], A. Lopez Alonso[1,2], B. Jose[2], N. Gilmartin[2], P. McCluskey[2], B. Byrne[2], A. Ricco [2,3], D. Kenny [1,2].
[1] Royal College of Surgeons in Ireland, Dublin, Ireland, [2] Biomedical Diagnostics Institute, Dublin City University, Ireland, and [3] NASA Ames Research Center, California, USA.

C5 14.45

AN ANIMAL MODEL FOR THE INVESTIGATION OF THE MECHANISM OF ACTION OF SACRAL NEUROMODULATION

J. Evers [1], E.V. Carrington[1,2], S.M. Scott SM, C.H. Knowles[3], P.R. O'Connell [1,4,] , J.F.X. Jones[1].

[1]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, [2]GI Physiology Unit, The Wingate Institute of Neurogastroenterology, Queen Mary, University of London, London, United Kingdom, [3]National Centre for Bowel Research and Surgical Innovation, Queen Mary, University of London, London, United Kingdom, [4]Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland.

C6 15.00

LIPOXIN A_4 REDUCES cAMP DEPENDENT ION TRANSPORT IN T84 CELLS

M.I. Hollenhorst[1,2], V. Urbach[1].

[1]National Children's Research Centre, Dublin, Ireland; [2]Royal College of Surgeons, Dublin, Ireland.

C7 15.15

NOVEL ROLE OF PREGNANCY SPECIFIC GLYCOPROTEINS IN PLATELET INTEGRIN BINDING

K. Golla[3], D.K. Shanley[1], P.A. Kiely[2], S. Allen[3], R.T. O'Riordan[1], N. Moran[3], T. Moore[1].

[1]Department of Biochemistry, University College Cork, Cork, Ireland, [2]Department of Life Sciences, and Materials and Surface Science Institute, University of Limerick, Limerick, Ireland, and [3]Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland.

C8 15.30

LIPOXIN STIMULATED EPITHELIAL REPAIR IS MEDIATED THROUGH POTASSIUM CURRENTS

P.J. Buchanan[1,2], P. McNally[1], V. Urbach[1,2,3].

[1]National Children's Research Centre, Our Lady's Children Hospital, Dublin 12, Ireland, [2]Department of Molecular Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland, and [3]INSERM U845, Faculté de Médecine, Paris, France.

Barcroft Medal Competition 15.45-16.45
Communications B1-B3

B1 15.45

VISCERAL PAIN SENSITIVITY AND STRESS-INDUCED DEFECATION IN THE WISTAR KYOTO RAT MODEL OF IBS IS SUPPRESSED BY INHIBITION OF INTERLEUKIN-6 SIGNALLING

M.M. Buckley[1,2] , D. O'Malley[2].

[1]Alimentary Pharmabiotic Centre and [2]Department of Physiology, University College Cork, Cork, Ireland.

B2 16.05

PLATELETS ENHANCE INVASION OF OVARIAN CANCER CELLS IN AN EXPERIMENTAL METASTASIS MODEL

N.M. Cooke[1,3], K. Egan[1,3], C. Spillane[2,3], J. O'Leary[2,3], D. Kenny[1,3].
[1]Department of Molecular and Cellular Therapeutics, RCSI, Dublin 2, [2]Department of Histopathology, Trinity College Dublin, and [3]The Biomedical Diagnostics Institute, Dublin City University, Dublin, Ireland.

B3 16.25

HIGHLY EFFICIENT NON-VIRAL GENE-ACTIVATED MATRICES INCORPORATING ANGIOGENIC AND OSTEOGENIC GENES ENHANCES BONE TISSUE REGENERATION IN VIVO

E.G. Tierney[1,2], C.M. Curtin[1, 2], S.A. Cryan[3], G.P. Duffy[1,2], F.J. O'Brien[1, 2].
[1]Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, [2]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland, and [3]School of Pharmacy, Royal College of Surgeons in Ireland, Dublin, Ireland.

Poster Viewing 16.45-17.15
Authors to attend. Tea/Coffee will be served.

Conway Review Lecture 17.15

Professor John F. Cryan, University College Cork

A gut feeling about brain function.

Presentation of Prizes 18.15

CONFERENCE DINNER 19.30

Posters
P1-P40

P1

HAWTHORN JUICE AND BLUEBERRY JUICE INCREASE HYDROGEN SULFIDE PRODUCTION IN RAT KIDNEY *IN VITRO*

M. Hynes*, A. L. Ní Churraoin*, L. A. Horrigan
Physiology, School of Medicine, National University of Ireland, Galway, Ireland.
* Equal contributors to the study

P2

LIPOXIN A_4 DELAYS THE INVASION OF CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS BY THE PATHOGEN *PSEUDOMONAS AERUGINOSA*

G. Higgins[1,2], P. McNally[1], V. Urbach[1,2]
[1]National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland, and [2]Royal College of Surgeons in Ireland, Dublin, Ireland.

P3

A COMPARISON OF BEHAVIOURAL PROFILES BETWEEN COMMERCIALLY OBTAINED AND IN-HOUSE BRED RATS.

K. Bannerton, K. McCabe, Z. McAleavey, J.P. Kelly.
Discipline of Pharmacology and Therapeutics, School of Medicine, NUI Galway, Ireland.

P4

THE ROLE OF DNA METHYLATION IN TOLL-LIKE RECEPTOR 3 EXPRESSION

O'Connell, S, McKernan, D.P .
Pharmacology and Therapeutics, NUI Galway, Ireland.

P5

RETROSPECTIVE STUDY OF BONE MINERAL DENSITY IN WOMEN WITH CHRONIC HEPATITIS C INFECTION IN CORK UNIVERSITY HOSPITAL

T. Jayaraman, S. Corbett, E. Kenny-Walsh, O. Crosbie.
Department of Hepatology, Cork University Hospital, Cork, Ireland.

P6

GENE THERAPY FOR MUSCULAR DYSTROPHY IN AN MDX MOUSE MODEL

S.D. McCarthy, K.J.A. McCullagh.
Department of Physiology, School of Medicine, and Regenerative Medicine Institute, National University of Ireland, Galway, Ireland.

P7

CENTRAL INHIBITION OF FATTY ACID AMIDE HYDROLASE ATTENUATES TLR-3 INDUCED EXPRESSION OF INTERFERON-GAMMA AND RELATED GENES IN THE RAT HIPPOCAMPUS

R. Henry[1,3], D. Kerr[1,2,3], D.P. Finn[2,3], M. Roche[1,3]
[1]Physiology and [2]Pharmacology and Therapeutics, School of Medicine, [3]NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland, Galway, Ireland.

P8

THE NEURODEVELOPMENTAL AND BEHAVIOURAL EFFECTS OF METHAMPHETAMINE EXPOSURE DURING PREGNANCY IN RATS

K. McDonnell Dowling, V. MacEoin, S. Walsh, S. O'Brien, J.P. Kelly.
Discipline of Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, Ireland.

P9

DEVELOPMENT OF MINIATURIZED BIOMEDICAL SENSOR DEVICES

W. Messina[1], E. J. Moore[1,2]
[1]Sensing and Separation Group, Chemistry Department and Life Science Interface Group, Tyndall National Institute, University College Cork, Cork, and
[2]Department of Chemistry, University College Cork, Cork, Ireland.

P10

RESOLVIN D1 (RVD1) INCREASES THE AIRWAY SURFACE LIQUID HEIGHT IN CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS

A. Moukachar[1,2], F. Ringholz[1], G. Higgins[1], V. Urbach[1].
[1]National Children's Research Centre, Crumlin, Dublin, Ireland, and [2]Université Pierre-et-Marie-Curie (UPMC), Paris VI, France.

P11

CROSSTALK BETWEEN GLP-1 AND IL-6 IN ISOLATED RAT MYENTERIC AND SUBMUCOSAL NEURONS

A. Kelliher, L. O'Sullivan, M. Buckley, D. O'Malley.
Department of Physiology, University College Cork, Cork, Ireland.

P12

THE EFFECT OF FOAM ROLLING ON SUBSEQUENT EXERCISE PERFORMANCE IN MAN

J. Bradley[1], J. Gomez[2], T. Woods[3].
[1]School of Education, [2]Mardyke Arena and [3]Department of Sport & Physical Activity, University College Cork, Cork, Ireland.

P13

SMALL-MOLECULE INHIBITORS AT THE PSD-95/nNOS INTERFACE HAVE ANTIDEPRESSANT-LIKE PROPERTIES IN MICE

M. V. Doucet[1,2], H. Levine[1], K. K. Dev[2], A. Harkin[1].

P14

BREAST CANCER AND DUCTAL CARCINOMA *IN SITU* IDENTIFICATION USING A DUAL-ELECTRODE SYSTEM

N.T.P. Savage[1], B.D. O'Donnell[2], M.J. O'Sullivan[2], E.J. Moore[1]
[1]Sensing and Separation Group, Department of Chemistry and Life Science Interface Group, Tyndall National Institute, University College Cork and [2]BreastCheck and Cork University Hospital, Cork, Ireland.

P15

CELL BASED BIOSENSORS FOR USE IN CYTOTOXICITY STUDIES

M.Fitzgerald[1], E.J. Moore[1]
[1]Sensing and Separation Group, Department of Chemistry and Life Science Interface Group, Tyndall National Institute, University College Cork, Ireland.

P16

EVIDENCE FOR THE INVOLVEMENT OF CYCLIC ADP RIBOSE-DEPENDENT ENDOPLASMIC RETICULUM Ca^{2+} RELEASE BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS.

J. Leahy, A. Kaar, M.G. Rae
Department of Physiology, University College Cork, Cork, Ireland.

P17

ESTABLISHMENT OF THE GUT MICROBIOME DURING EARLY LIFE INFLUENCES THE SURVIVAL OF NEWLY-BORN CELLS IN THE ADULT HIPPOCAMPUS

S. Ogbonnaya[1,3], G. Clarke[2,4], F. Shanahan[2], J.F. Cryan[1,2], O.F. O'Leary[1].
[1]Department of Anatomy and Neuroscience, [2]Alimentary Pharmabiotic Centre, University College Cork, [3]Department of Neurosurgery, Cork University Hospital, Ireland, and [4]Department of Psychiatry, University College Cork, Ireland

P18

THE IMPACT OF HIGH SALT DIET ON THE REGULATION OF RENAL HEMODYNAMICS AND NITRIC OXIDE IN WISTAR RATS

H. F. Shabana, A. F. Ahmeda, E. J. Johns.
Department of Physiology, University College Cork, Ireland.

P19

PROLIFERATION OF HEPATIC STELLATE CELLS FOLLOWING TRANSPLANTATION OF THE LIVER: AN INTRAVITAL FLUORESCENCE MICROSCOPY STUDY

R. C. Chigozie[1], C. Prendergast[1], M. Tawadrous[2], A.M. Wheatley[1,2].
[1]Department of Physiology, School of Medicine, NUI Galway, Ireland and [2]Department of Physiology, University of Otago, New Zealand.

P20

A PROSPECTIVE AUDIT OF HAEMATINIC REQUESTS FROM GENERAL PRACTICE IN CORK UNIVERSITY HOSPITAL (CUH) HAEMATOLOGY LABORATORY

M. O'Meara[1], S. Cadogan[2], M. O'Reilly[2], D. Minihane[2], M.R. Cahill[2]
[1]Department of Biochemistry, St James' Hospital, Dublin 8 and [2]Department of Haematology, Cork University Hospital, Cork, Ireland.

P21

BILATERAL MICROINJECTION OF THE CB1 RECEPTOR AGONIST ACEA INTO THE PAG DIFFERENTIALLY MODULATES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR IN SPRAGUE-DAWLEY AND STRESS HYPER-RESPONSIVE WISTAR-KYOTO RATS

E. M. Jennings [1,2] , K. Rea [1,2] B. Okine [1,2], F. McGowan[1], M. Roche [2,3], D.P. Finn [1,2]
[1] Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, [2] NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland, Galway, and [3]Physiology, School of Medicine, National University of Ireland, Galway, Ireland.

P22

INHIBITORY ACTION OF THE TRPA1 ANTAGONIST, HC-030031, ON RAT TRPM8

C. Doran, G. Reid.
Department of Physiology, University College Cork, Ireland.

P23

CAMPHOR ACTIVATES AND SENSITIZES RAT AND HUMAN TRPM8 TO ICILIN

T. Selescu[1,2], C. Ciobanu[1,2], C. Dobre[1], G. Reid[2], A. Babes[1].
[1]Department of Anatomy, Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania and [2]Department of Physiology, University College Cork, Ireland.

P24

CHICK, BUT NOT RAT, SENSORY NEURONES EXPRESS THE WARM-ACTIVATED ION CHANNEL TRPV3

C. Doran, N. Morshed, G. Reid
Department of Physiology, University College Cork, Ireland.

P25

TRPV1 BLOCKADE RESTORES THE CARDIOPULMONARY BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY IN CISPLATIN-INDUCED RENAL FAILURE RATS

M. Duff[1], M.H. Abdulla[1], E. J. Johns[1,2]. [1]Department of Physiology, University College Cork, Ireland and [2]Allianze University, College of Medical Sciences, Penang, Malaysia.

P26

THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA CO-ACTIVATOR 1-ALPHA IN PREGNANCY

A.C. Delany[1], S.K. Walsh[2], L.C. Kenny[3], F.P. McCarthy[3].
[1]Department of Physiology, University College Cork, [2]The Robert Gordon University, Aberdeen, UK, and [3]The Irish Centre for Fetal and Neonatal Translational Research, Department of Obstetrics & Gynaecology, University College Cork, Ireland.

P27

NOVEL INTERACTIONS OF SUCCINATE, LIPOPOLYSACCHARIDE AND HYPOXIA ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS IN VITRO

L.M. Alvey, J.J. O'Connor.
UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

P28

ACUTE HYPOXIC PRE-CONDITIONING AND PROLYL-HYDROXYLASE INIBITION IMPROVES SYNAPTIC TRANSMISSION RECOVERY TIME FROM A SUBSEQUENT HYPOXIC INSULT IN THE RAT HIPPOCAMPUS

A.E. Corcoran, J.J. O' Connor.
UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

P29

A NOVEL ROLE FOR ADRENERGIC AND SEROTONERGIC SIGNALING IN THE ACTION OF ERGOMETRINE IN ISOLATED TERM HUMAN MYOMETRIUM

C. Leyden[1], R.A. Fanning[2], D.P. Campion[3], J.J. O'Connor[1].
[1]UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, [2]Department of Perioperative Medicine, Coombe Women and Infant's University Hospital, Dublin, and [3]UCD School of Veterinary Medicine, University College Dublin, Ireland.

P30

EFFECTS OF CHRONIC INTERMITTENT HYPOXIA AND PROLYL-HYDROXYLASE INHIBITION ON SYNAPTIC TRANSMISSION AND PLASTICITY IN THE RAT CA1 AND DENTATE GYRUS

A. Wall[1], A.E. Corcoran[1], K.D. O'Halloran[2], J.J. O' Connor[1].
[1]UCD School of Biomolecular and Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland and [2]Department of Physiology, School of Medicine, University College Cork, Ireland.

P31

XANTHINE OXIDASE INHIBITION IMPROVES RAT PHARYNGEAL DILATOR MUSCLE FUNCTION IN VITRO

D. Burns, P. Lewis, K.D. O'Halloran
Department of Physiology, School of Medicine, University College Cork, Ireland.

P32

AN INVESTIGATION OF THE EFFECT OF GREEN TEA EXTRACT (CAMELLIA SINENSIS) SUPPLEMENTATION ON FAT METABOLISM IN EXERCISING HUMANS

M. Corcoran[1], J. Bradley[2], T. Woods[3], K.D. O'Halloran[1].
Departments of [1]Physiology, [2]Education and [3]Sport and Physical Activity, University College Cork, Cork, Ireland.

P33

EFFECTS OF NEONATAL EXPOSURE TO CHRONIC INTERMITTENT HYPOXIA ON RESPIRATORY MUSCLE FUNCTION AND REDOX STATUS

R. Williams[1], F. McDonald[3], V. Healy[1], D. Sheehan[2] and K.D. O'Halloran[1]
Departments of [1]Physiology and [2]Biochemistry, University College Cork, and [3]School of Medicine and Medical Science, Health Sciences Centre, University College Dublin, Ireland.

P34

CHRONIC INTERMITTENT HYPOXIA INCREASES NADPH OXIDASE SUBUNIT EXPRESSION IN RAT STERNOHYOID MUSCLE

R. Williams[1], E. Lucking[3], D. Sheehan[2], V. Healy[1], K.D. O'Halloran[1]
Departments of Physiology[1] and Biochemistry[2], University College Cork, Ireland and [3]School of Medicine and Medical Science, Health Sciences Centre, University College Dublin, Dublin, Ireland.

P35

DIAPHRAGM MUSCLE REMODELLING IN A RAT MODEL OF CHRONIC INTERMITTENT HYPOXIA

C. Shortt[1], A. Fredsted[2], A. Bradford[3], J.F.X. Jones[1], K.D. O'Halloran[4].
[1]UCD School of Medicine and Medical Science, University College Dublin, Ireland, [2]Department of Biomedicine, Aarhus University, Denmark, [3]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, and [4]Department of Physiology, School of Medicine, University College Cork, Ireland.

P36

NEONATAL EXPOSURE TO CHRONIC INTERMITTENT HYPOXIA CAUSES PERSISTENT RESPIRATORY MUSCLE WEAKNESS IN MALE AND FEMALE RATS

F.B. McDonald[1], D. Edge[1], K.D. O'Halloran[2].
[1]UCD School of Medicine and Medical Science, University College Dublin and
[2]Department of Physiology, School of Medicine, University College Cork, Ireland.

P37

EFFECT OF CHRONIC INTERMITTENT HYPOXIA ON THE REFLEX RECRUITMENT OF THE GENIOGLOSSUS DURING AIRWAY OBSTRUCTION IN THE ANAESTHETISED RAT

D. Edge[1], F.B. McDonald[1], J.F.X. Jones[1], A. Bradford[2], K.D. O'Halloran[3].
[1]UCD School of Medicine and Medical Science, University College Dublin,
[2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, and [3]Department of Physiology, School of Medicine, University College Cork, Ireland.

P38

THE EFFECTS OF VARIATIONS IN THE N-TERMINUS ON THE SENSITIVITY OF CHICK TRPM8 TO MENTHOL AND COLD

N. Morshed, G. Reid
Department of Physiology, University College Cork, Ireland.

P39

REDUCED 15-LO2 AND LIPOXIN A_4 / LEUKOTRIENE B_4 RATIO IN LOWER AIRWAYS OF CHILDREN WITH CYSTIC FIBROSIS

F.C Ringholz, P. McNally, V. Urbach.
National Children's Research Centre, Crumlin, Dublin, Ireland.

P40

DISCOVERY OF CADHERIN-5 PROTEIN IN HUMAN PLATELETS

Golla. K[1] .,Treumann, A[3] ., Shields. D[2]., Moran. N[1]

[1]Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, [2]Complex and Adaptative systems Laboratory (CASL), University College Dublin, Ireland, and [3]Biopharmaceutical Bioprocessing Technology Centre, Newcastle University, UK.

Abstracts

D1

INVESTIGATING THE EFFECTS AND PUTATIVE MECHANISM OF ACQUIRED RESISTANCE TO NERATINIB IN BREAST CANCER

S. Breslin, C. Corcoran, S. Rani, L. O' Driscoll.
School of Pharmacy and Pharmaceutical Sciences & Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland.

HER2-overexpression occurs in 20-25% of breast cancer cases and is commonly treated with HER2-targeting drugs. While development of targeted therapy has greatly improved the outcome for HER2-positive patients, issues due to intrinsic/acquired resistance remain problematic. Neratinib is an irreversible EGFR, HER2 and HER4 tyrosine kinase inhibitor currently in phase III clinical trials. We have developed 2 novel neratinib-resistant (NR) breast cancer cell line variants to serve as *in vitro* models of drug-resistance. Using these models, we investigated if resistance to neratinib is associated with cross-resistance to other anti-cancer drugs, phenotypic changes within cells and explored a potential mechanism of resistance to neratinib in a step towards its circumvention.

NR cell line variants were developed by exposing HER2-overexpressing breast cancer cell lines, HCC1954 and SKBR3, to increasing concentrations of neratinib over several months. Proliferation assays were used to establish IC_{50} values for neratinib in NR and aged-parent cells; as well as to assess possible cross-resistance to lapatinib, afatinib and docetaxel. Phenotypic changes in NR cells were examined using migration, invasion and *anoikis* assays. Immunoblotting was performed to discover protein changes directly associated with neratinib-resistance. All assays n=3, minimum.

HCC1954-NR and SKBR3-NR cell variants were 6.5±0.4 and 194±47-fold more resistant to neratinib than aged-parent cells, respectively. Furthermore, HCC1954-NR variants were cross-resistant to afatinib (37±7.23-fold) and lapatinib (10±0.8-fold). SKBR3-NR variants were cross-resistant to afatinib by >163.3±22.7-fold (IC_{50} not reached at 9μM afatinib where, on average, 65.5% cell growth is maintained) and lapatinib (162.3±22-fold). No cross-resistance to docetaxel was observed. Cells with acquired neratinib-resistance were found to be more migratory, invasive and resistant to *anoikis* than aged-parent cells. NR cells demonstrated increased IGF1R, Hsp90 and Bcl2 protein expression and decreased caspase-8 levels, in comparison to aged-parent cells. Consequently, treatment of HCC1954-NR cells with NVP-AEW541 (an IGF1R-inhibitor) in addition to neratinib yielded more cell death (9%) than with neratinib treatment alone.

Chronic exposure of breast cancer cells to neratinib results in acquired resistance to the drug. Neratinib-resistance conferred cross-resistance to other HER2-targeting drugs and induced a more aggressive phenotype in these cells. Altered expression of IGF1R, Hsp90, Bcl2 and caspase-8 indicate that these proteins are involved in the pathway of neratinib-resistance. To date, this mechanism of resistance has not previously been associated with HER2-targeted therapy. Further investigation into the potential relevance of co-treatment with neratinib and Hsp90- or Bcl2-inhibitors are now warranted.

The authors acknowledge the Marie Keating Foundation PhD Scholarship at TCD, Trinity Foundation & SFI (08/SRC/B1410).

D2

CLOSING A GAP IN SKELETAL MUSCLE EXCITATION-CONTRACTION COUPLING: THE 90 kDa JUNCTIONAL FOOT PROTEIN IS JUNCTOPHILIN

K.K. English[1], A. Carla[2], V. Sorrentino[2], J.J. Mackrill[1].
[1] Department of Physiology, University College Cork, Ireland and
[2] Molecular Medicine Section, Department of Neuroscience and Interuniversity Institute of Myology, University of Siena, Siena, Italy.

In skeletal muscle excitation-contraction coupling (ECC), the triad junctions represent the site of communication between the t-tubules and terminal cisternae of the sarcoplasmic reticulum. During the late 1980's, Campbell *et al* (1) generated of a panel of antibodies against skeletal muscle triad proteins, thus providing a key source of immunological reagents for the characterisation of components involved in ECC. One clone from this library of monoclonal antibodies, mAb VF1c, recognised a constituent of 90 kDa apparent molecular weight, and subsequent studies indicated that this protein, termed the 90 kDa junctional sarcoplasmic reticulum protein (JSR90) or the 90 kDa junctional foot protein (JFP90), formed part of a macromolecular complex with the RyR, indicating that it plays a role in ECC (2).

Despite its potential importance in ECC, the molecular identity of JFP90 has not been determined. In the current study, in order to address this deficiency, JFP90 was immunoprecipitated using mAb VF1c from solubilised rabbit skeletal muscle as two major bands, of 90 kDa and 68 kDa. Proteomic analyses of these bands indicated that they are full length junctophilin-1 (JPH1) and a truncated form of this protein. JPH1 is a triad enriched protein that spans the junctions between the t-tubules and terminal cisternae (2) and which interacts with the RyR calcium release channel. Probing a set of JPH1 fragments heterologously expressed in yeast indicates that the epitope recognised by mAb VF1c resides between residues 369 and 460.

Taken together with recent findings which implicate JPH1 in processes as diverse as sarcopenia, fibre-type switching and muscular dystrophy, this information should prove invaluable to further the understanding of its role in ECC in health and disease.

References

1. Campbell KP, Knudson CM, Imagawa T, Leung AT, Sutko JL, Kahl SD, et al. Identification and characterization of the high affinity [^{3}H]ryanodine receptor of the junctional sarcoplasmic reticulum Ca^{2+} release channel. *The Journal of biological chemistry* (1987) 262(14):6460–3.

2. Golini L, Chouabe C, Berthier C, Cusimano V, Fornaro M, Bonvallet R, et al. Junctophilin 1 and 2 interact with the L-type Ca^{2+} channel dihydropyridine receptors (DHPRs) in skeletal muscle. *The Journal of biological chemistry* (2011)286(51)**:**43717–25.

D3

INVESTIGATING THE MOLECULAR MECHANISMS THAT UNDERPIN MOUSE RESPIRATORY MUSCLE ADAPTATION TO CHRONIC HYPOXIA: A REDOX PROTEOMICS APPROACH

P. Lewis[1], D. Sheehan[2], K.D. O'Halloran[1].
Departments of [1]Physiology and [2]Biochemistry, University College Cork, Ireland.

Chronic hypoxia (CH) induces respiratory muscle functional remodelling similar to chronic obstructive pulmonary disease which may be related to alterations in redox homeostasis. This remodelling is time-dependent and differential to limb muscle. We hypothesized that CH induces differential protein oxidation in respiratory and limb muscle and that this phenomenon is dependent on the length of exposure to hypoxia.

C57Bl6J mice were exposed to 1, 3 and 6 weeks of CH (10% F_iO_2) or normoxia. Respiratory and limb muscle homogenates were incubated with carbonyl- or thiol-reactive fluorophores before 1D/2D-PAGE and fluorescence scanning. Significantly modified proteins were identified by mass spectrometry (MS).

Increased carbonyl content and decreased free thiol content was observed after 6 weeks of CH in the respiratory muscles (carbonyl and free thiol change in diaphragm and sternohyoid respectively; $P<0.0001$, $P<0.01$, $P<0.0001$and $P<0.01$; n=7-8 per group; Student's t-test) in contrast to significant increases in free thiol content in the limb muscles (EDL, soleus; $P<0.001$, $P<0.0001$). One week of CH resulted in no change in carbonyl content but a significant increase in free thiol content in the diaphragm ($P<0.001$). Furthermore, catalase activity in the diaphragm was significantly increased after 1 week of CH and maintained after 3 and 6 weeks of CH ($P<0.001$, $P<0.05$ and $P<0.05$ respectively) suggesting an early antioxidant response that is ultimately overwhelmed. Protein-specific changes in the respiratory muscles after 6 weeks of CH were identified using 2D-redox proteomics and MS. Proteins regulating metabolic substrate flux, Ca^{2+} handling, iron and pH homeostasis and various signalling pathways are differentially modified in diaphragm and sternohyoid muscle. Differential protein expression changes were also observed.

We have shown that prolonged exposure to CH causes an accumulation of protein oxidation in respiratory muscles, differential to limb, that may contribute to changes in respiratory muscle homeostasis and function. An early and maintained increase in antioxidant defence is insufficient to prevent this. The cellular location of redox modified proteins suggests the mitochondria as the principle site of the stress but redox modulation was also observed in proteins of cellular organelles and the cross-bridge. Redox proteomics is a powerful tool for identifying redox post-translational modifications in the muscle proteome. Redox modulation likely underpins functional plasticity in the respiratory muscles after CH exposure.

Funded by the Health Research Board, Ireland and the Strategic Research Fund, University College Cork, Ireland.

D4

^{13}C-GLUCOSE BREATH TEST IN THE DIAGNOSIS OF DIABETES IN HUMANS

L. Lynch, A. Shafat.
Physiology, School of Medicine, National University of Ireland, Galway, Ireland.

Current diagnostic criteria for type 2 diabetes mellitus (TIIDM) rely on increased blood glucose concentration following an oral glucose tolerance test (OGTT) or increased glycated haemoglobin (HbA_{1c}). Changes in these parameters occur long after insulin resistance (IR) manifests as plasma glucose concentration is homeostatically defended. In contrast, glucose oxidation measured by the ^{13}C-glucose breath test (^{13}C-GBT) is reduced as a direct consequence of IR. Therefore the aim of this study was to determine the clinical applicability of the ^{13}C-GBT as a diagnostic tool for TIIDM.

Twenty three TIIDM {62.7(11.6)yr, 92.7(13.1)kg, 173.6(6.9)cm and twenty three controls {50.9(10.1)yr, 86.1(9.4)kg, 179(7.0)cm, all data mean (SD)} underwent simultaneous OGTT and ^{13}C-GBT following a 12hr overnight fast. Fasting samples were obtained for plasma glucose, insulin, c-peptide, leptin, adiponectin, HbA_{1c} and two breath $^{13}CO_2$ abundance values (at t=-15and-5min). At t=0min, each subject drank a 75g glucose solution (Thornton & Ross, UK) containing 0.15g of 1-^{13}C glucose. Further breath (every 15min over a 240min interval) and further plasma blood glucose, insulin and c-peptide samples (90,120,180min) were also taken. Differences were examined between TIIDM and controls using independent Student's t-test. Pearson's coefficient correlations were examined between the ^{13}C-GBT and IR measures.

Peak increase in $^{13}CO_2$ breath enrichment (DOB) and per cent dose recovered (PDR) were significantly reduced in TIIDM compared to controls ($P<0.01$). The ^{13}C-GBT correlated well with measures of IR. Pearson's coefficient correlations were observed between PDR_{240} and fasting plasma glucose (FPG) ($r=-0.481, P<0.01$), 2hr plasma glucose (2hrPG) ($r=-0.655, P<0.01$), homeostasis model of assessment-IR (HOMA-IR) ($r=-0.385, P<0.01$), quantitative insulin sensitivity check index (QUICKI) ($r=0.346, P<0.05$), oral glucose insulin sensitivity index (OGIS) ($r=0.497, P<0.01$) and HbA_{1c} ($r=-0.455, P<0.01$) and Peak DOB and FPG ($r=-0.367$ $P<0.05$), 2hrPG ($r=0.401, P<0.01$), HOMA-IR ($r=-0.374, P<0.05$), QUICKI ($r=0.398, P<0.05$) and OGIS ($r=0.394, P<0.01$).

The ^{13}C-GBT measures whole body glucose oxidation, correlates well with other indices of IR and differentiates between TIIDM and controls demonstrating its potential to be developed as a diabetes diagnostic test.

Research was approved by the Clinical Research Ethics Committee, Galway University Hospitals. Ref: C.A.658.

D5

GASTROINTESTINAL CONTRACTILE ACTIVITY IN THE *MDX* MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY, A ROLE FOR IL-6 IN PATHOPHYSIOLOGY

J. Manning, D. O' Malley.
Department of Physiology, University College Cork, Cork, Ireland.

Duchenne Muscular Dystrophy (DMD) is characterised by severe progressive wasting of skeletal muscle and shortened life expectancy. Deficiency of the protein dystrophin causes a chronic inflammatory response and eventual muscle fibre degeneration. The myokine - interleukin-6 (IL-6) is elevated in patients with DMD. Dystrophin is absent from smooth muscle cells; and a little-reported symptom of DMD is altered gastrointestinal (GI) function. Patients suffer from bloating, constipation and case reports of acute gastric dilation and intestinal pseudo-obstruction. The aim was to investigate IL-6 and the stress hormone, corticotropin-releasing factor (CRF) in *mdx* mice to determine if elevation of IL-6 and possible interactions with CRF are associated with (GI) pathophysiology.

Morphological changes were investigated in Haematoxylin & Eosin stained colonic sections. To investigate GI function, colon sections from wildtype (WT) and *mdx* (C57BL/10ScSn-Dmdmdx/J) mice (10 weeks old), suspended in Krebs buffer in carbogenated organ baths, to compare contractile activity under basal conditions and following stimulation with IL-6 (200nM) and/or CRF (200nM).

In studies investigating stress-induced defecation, mdx mice exhibited lower transit times in every 15 minute increment and cumulatively over a 90 minute trial ($n=12$, $p<0.05$). Histologically, *mdx* mice exhibit a thickened muscular layer ($141\pm7.918\mu m$, $n=4$) compared to WT mice ($95.26\pm7.494\mu m$, $n=4$, $p<0.001$). The mucosal layer and colonic diameter was unchanged from WT mice. Contractile activity was assessed in organ baths. IL-6 stimulated a six-fold greater contractile response in *mdx* ($172.6\pm 66.48\%$, $n=6$, $p<0.05$) compared to WT ($30.39\pm 6.512\%$, $n=7$) mice. Pre-incubation of the tissue with the Na^+ channel blocker, tetrodotoxin (TTX, 250nM) attenuated this response in *mdx* mice ($26.14\pm14.58\%$, $n=5$, $p=0.05$) but had no effect in the WT mice ($24.68\pm3.078\%$, $n=4$, $p>0.05$). In both strains ($n=6$, $p>0.05$) the stimulation of contraction by IL-6 and/or CRF was similar. However, the neuronally-mediated effects of IL-6 in *mdx* mice was borne out by the inhibitory effects of TTX on IL-6 plus CRF in *mdx* mice only ($1.681\pm0.6920\%$, $n=6$, $p<0.01$).

These data begin to elucidate the physiological GI changes in the dystrophin-deficient *mdx* mice which may contribute to altered function. These results indicate that neuronally regulated IL-6-induced muscular contraction is important in GI pathophysiology in this strain.

The authors acknowledge grant support from Muscular Dystrophy Ireland.

D6

OESTROGEN AND HYPOXIA MODULATE GENE EXPRESSION IN COLORECTAL CANCER CELLS THROUGH SPECIFIC MICRORNA REGULATION

Á. Nolan[1], I. Bray[2], R. Stallings[2], A. Silver[3], W. Thomas[1], B. Harvey[1].
Departments of [1]Molecular Medicine and [2]Cancer Genetics, Royal College of Surgeons in Ireland, Dublin, Ireland and [3]Colorectal Cancer Genetics, Barts and the London School of Medicine and Dentistry, London, UK.

Women experience a lower incidence of colorectal cancer (CRC) than men, and oestrogen receptor beta (ERβ) expression by CRC cells is associated with improved patient survival. The role of ERs in CRC is controversial and the effect of oestrogen in the depleted oxygen environment of the tumour has not been investigated. MicroRNAs (miRNAs) contribute to changes in gene expression detected in oncogenesis and are regulated by oestrogen in other malignancies. In this study we identified 17β-oestradiol (E2)-regulated miRNAs and their mRNA targets in CRC under conditions of normoxia and hypoxia, and established the receptor coupling of the oestrogen action.

CRC cell lines (HT29, DLD1, HCT116, SW837, C80, C99 and HT55) were cultured in normoxia (20% O_2) or hypoxia (2% or 0.2% O_2). ER abundance was analysed by RT-qPCR and Western blot. E2 (10nM, 24h)-responsive miRNAs and mRNAs were determined by array analyses of 750 miRNAs and 24,000 mRNAs. Validation of E2-responsiveness was confirmed by RT-qPCR and mimicked by treatment with ER-specific agonists. Target Scan (www.targetscan.org) was used to predict mRNA targets for the miRNAs. Cell line gene expression was correlated with human colonic tumour specimens by meta-analysis of the R2 microarray and visualisation platform (http://r2.amc.nl). *In vitro* scratch assays were used to evaluate cell migration. All experiments were performed in biological triplicate. Statistical significance was determined by Students t-test.

ERα and ERβ were not expressed however the G-protein coupled ER (GPER) was present in CRC cell lines and tumour specimens and GPER expression was hypoxia-induced in HT29 cells at 2% (5-fold, $p \leq 0.01$) and 0.2% (6.5-fold, $p \leq 0.001$) O_2. Twelve E2-responsive miRNAs were identified in HT29 cells (>2-fold, $p \leq 0.05$), including induction of the oncogenic miR-17-92 cluster. MiR-20a was the most highly induced cluster member (3.4-fold, $p=0.002$). E2 treatment repressed the expression of 153 mRNAs and induced 197 mRNAs (>1.5-fold, $p \leq 0.05$). E2-repressed mRNAs included *ATM*, a known modulator of tumourigenesis. E2-induced mRNA repression was mimicked by the GPER agonist G1 (1μM, 24h). Novel E2-induced miRNAs and predicted E2-repressed target mRNAs, including miR-20a and *ATM*, were hypoxia-responsive in CRC cell lines and E2 treatment (10nM, 24h) potentiated HT29 cell migration by 10.4% in 2% O_2 ($p=0.03$).

MiRNA induction through GPER may contribute to tumour promotion in CRC following loss of ERβ and is sensitive to O_2 partial pressure. The dichotomous oestrogen actions may be the basis for the controversies surrounding the effects of HRT on CRC progression.

The authors acknowledge funding from the HRB.

D7

THE EFFECTS OF HYPOXIA-MIMETIC DRUGS ON SYNAPTIC TRANSMISSION AND SYNAPTIC PLASTICITY IN THE DENTATE GYRUS IN VITRO

H. Nolan, A.E. Corcoran, J.J. O'Connor.
UCD School of Biomolecular and Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

Hypoxia inducible factor (HIF) is a key regulator in oxygen homeostasis. Degradation of HIF under normoxic conditions is regulated by proline and asparaginyl hydroxylases. Inhibition of these enzymes is a novel target to modulate the hypoxic response for therapeutic benefit. Hypoxia-mimetic drugs, or prolyl-4-hydroxylase domain (PHD) enzyme inhibitors, are potential therapeutic agents for the prevention or treatment of stroke. Inhibition of PHDs has been shown to delay cell death, and protect against ischemic injury in the hippocampus[1]. This study examined the effects of three such inhibitory agents, dimethyloxalyl glycine (DMOG), JNJ-42041935 (both of which act as competive antagonists of the 2-oxoglutarate binding site) and deferoxamine (DFO; prevents PHD activation by the removal of Fe^{++}) on synaptic transmission and synaptic plasticity in the rat dentate gyrus. A very recent publication from this laboratory has reported modulatory effects of PHD inhibition on synaptic plasticity in the CA1 hippocampal region[2].

Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the medial perforant pathway in isolated slices of the dentate gyrus of the hippocampus. Long-term potentiation (LTP) was induced by high frequency stimulation with 3 trains separated by 20 s (each train of 1 s at 100Hz) in the presence of 100 μM picrotoxin. All data are presented as mean±sem and compared using the Student's t-test.

Application of DMOG (1 mM), but not DFO (10 μM) or JNJ (10 μM) resulted in a small but significant decrease in fEPSP amplitude (76.9±6.0%; n=5; $P<0.05$ for DMOG versus control of 96.9±3.0%). Further experiments using isolated NMDAR-mediated fEPSPs showed that the each of the three inhibitors did not alter the amplitude of NMDA fEPSPs. Application of all compounds caused a significant reduction in LTP (88.7±13.5; 90.1±12.9%; 107.4±2.7% respectively versus controls of 135.1±8.3%; $P<0.05$ for all three at 60 min post tetanus).

These results indicate that the inhibition of LTP in the dentate gyrus by PHD inhibition is modulated by a mechanism which does not involve NMDA receptors. Furthermore we have provided novel evidence, which implicates a role for PHD enzymes or HIF in this modulation of plasticity in the dentate gyrus.

References

1. Batti, L., Taylor, C.T., O'Connor J.J. (2010). Hydroxylase inhibition reduces synaptic transmission and protects against a glutamate-induced ischemia in the CA1 region of the hippocampus. *Neurosci,* 167, 1014-1024.
2. Corcoran, A., Kunze, R., Harney, S.C., Breier, G., Marti, H.H., O'Connor, J.J. (2013) A role for prolyl hydroxylase domain proteins in hippocampal synaptic plasticity. *Hippocampus, in press.*

D8

ALPHA-1 ANTITRYPSIN AS A POTENTIAL THERAPY TARGETING LEUKOTRIENE B_4 MEDIATED RESPIRATORY INFLAMMATION

C.A. O' Dwyer, N. Banville, N.G. McElvaney, E.P. Reeves.
Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland.

Neutrophil driven airway inflammation is a major factor in the pathology of chronic obstructive pulmonary disease (COPD) associated with alpha-1 antitrypsin (AAT) deficiency (AATD). The majority of individuals with severe AATD are homozygous for the Z allele, a risk factor for early onset of emphysema. Leukotriene B_4 (LTB_4) is a pro-inflammatory agent that rapidly activates neutrophils through its receptor BLT1, contributing to the neutrophil burden in the airways. The aim of this study was to investigate the ability of exogenous AAT to act as an LTB_4 antagonist. The biological consequence of the described AAT induced inhibition was investigated at the level of calcium (Ca^{2+}) flux and downstream signalling events including neutrophil adherence and release of proteolytic enzymes.

Asymptomatic AATD individuals were recruited from the Irish Alpha-1 Antitrypsin Deficiency Registry (n=15). Ethical approval was obtained from Beaumont Hospital Ethics Committee. Ca^{2+} and inositol triphosphate (IP_3) levels were assessed fluorometrically by stimulating neutrophils from healthy control individuals (n=15) with LTB_4 (100nM) in the presence and absence of AAT (27.5µM). Neutrophils were loaded with calcein-AM dye and adherence to fibronectin coated surfaces in response to LTB_4 and LTB_4 with AAT was examined. The effect of AAT on LTB_4 induced neutrophil degranulation was analysed by stimulating neutrophils with LTB_4 (100nM/ $2x10^7$cells/ml) in the presence of AAT (27.5µM). The interaction between LTB_4 and AAT was assessed spectrophotometrically.

Our *in vitro* data indicates that AAT prevents LTB_4 signalling, as indicated by the lack of cytosolic Ca^{2+} flux and IP_3 increase. Results demonstrate that the adhesive effect of LTB_4 is prevented in the presence of AAT ($P<0.05$) and that the levels of degranulated myeloperoxidase (MPO), hCAP-18 and matrix metalloprotease 9 (MMP-9) (markers of 1^o, 2^o and 3^o granule release respectively) were significantly decreased in the presence of AAT ($P<0.05$). The mechanism of inhibition involved direct binding of AAT to LTB_4 as deduced by reduction in vibrational fine structure indicating complexation of the two molecules. LTB_4 appears to bind to the hydrophobic pocket on the AAT molecule, as determined by competitive displacement assays with Gemfibrozil (0.25µM).

The results of this study indicate that AAT can inhibit LTB_4 signaling thereby reducing adhesion and the degranulation of proteolytic enzymes by neutrophils. This study proposes AAT aerosolized augmentation therapy as an effective treatment for LTB_4 associated pulmonary diseases.

D9

THE EXPRESSION OF NPY 1 RECEPTOR IN EXPERIMENTAL TEMPORAL LOBE EPILEPSY

E. O'Loughlin[1], K. McDermott[1] and D. Yilmazer-Hanke[1, 2].
[1] Department of Anatomy, University College Cork, Cork, Ireland and
[1,2] Department of Biomedical Sciences, Creighton University, Omaha, USA.

Temporal lobe epilepsy (TLE) is a chronic neurological condition that is characterised by reoccurring unprovoked seizures originating from the temporal lobe structures. The amygdala is known to play an important role in emotional behaviour and in epileptogenesis[1]. Neuropeptide Y (NPY) is an endogenous peptide with anti-convulsant properties. NPY 1 receptor (NPY1R) is widely expressed within the hippocampus and amygdala. It has been reported to be downregulated in anxiety and also by seizure activity[2].

To study TLE the kainic acid (KA) model was used. Stereotaxic surgery was carried out on 6-8 week old C57BL6j male mice. Seizures were induced by unilateral injection of kainic acid dissolved in saline (0.2 µg in 50 nL) into the right dorsal hippocampus. Control animals were injected with 0.9% saline under identical surgical conditions. Elevated plus maze (EPM) was carried out to assess anxiety-like behaviour in treatment groups. NPY1R ELISA was carried out on homogenized brain tissue. Brains were vibratomed and stained for cresyl violet, NPY1R and GFAP.

EPM data showed KA animals exhibited increased anxiety like behaviour in comparison to control animals. ELISA analysis showed hippocampal levels of Y1R were decreased in KA animals in comparison to control animals (*$p<0.05$). Histology results showed total ipsilateral hippocampal volume was significantly decreased in KA treated animals (*$p<0.05$). Ipsilateral CA1 region was decreased (*$p<0.05$). However, hilus volumes were increased in ipsi- and contralateral hippocampi (*$p<0.05$) in comparison to control animals. Total ipsilateral amygdala volume was decreased in KA animals (*$p<0.05$)**.** Volumes were altered in ipsilateral lateral (*$p<0.05$), Basolateral (*$p<0.05$) and central (**$p<0.01$) nuclei. Contralaterally, nuclear volumes in KA treated animals were similar to saline controls. NPY1R expression was decreased in ipsilateral CA1 (**$p<0.01$) and CA3 (*$p<0.05$) subregions and also in contralateral CA1 region (*$p<0.05$). NYP1R expression is decreased in ipsilateral granule layer of the dentate gyrus in KA treated animals in comparison to saline treated animals (**$p<0.01$). Pathology was further investigated using non-parametric spearman rank correlations between volume and GFAP intensity score in hippocampus and amygdala of KA and saline treated animals. However, no correlation was found.

Studies have shown that in TLE, the hippocampus and amygdala undergo irreversible anatomical rearrangements that often contribute to co-morbidity factors such as anxiety. In this animal model, we have shown that alterations occur in these structures at both molecular and cyto-architectural levels and that these changes may have a negative impact on anxiety-like behaviour.

The authors acknowledge grant support from the Health Research Board.

References

1. Pitkanen, A. and D.G. Amaral, Organization of the intrinsic connections of the monkey amygdaloid complex: projections originating in the lateral nucleus. J Comp Neurol, 1998. 398(3): p. 431-58.
2. Vezzani Annamaria, S.G., Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides, 2004. 38: p. 245-252

D10

DECREASED NEUTROPHIL MEMBRANE CHOLESTEROL CONTENT CAUSES LIPID RAFT STABILITY, IN PART EXPLAINING DYSREGULATED NEUTROPHIL ACTIVITY IN CYSTIC FIBROSIS

M. White, S. Cox, E. Hayes, N.G. McElvaney, E.P. Reeves.
Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland.

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CF is characterized by chronic bacterial infection of the airways and neutrophil dominated inflammation. CFTR is located in cholesterol rich lipid raft microdomains. Cholesterol, a structural component of lipid rafts, is involved in the trafficking of CFTR to lipid rafts and changes in cholesterol content can lead to dysregulated lipid raft structure. Considerable debate exists as to the underlying cause of dysregulated neutrophil activity in CF; therefore, the aim of this study was to investigate whether an intrinsic CFTR-related defect modifies neutrophil function or whether the inflammatory status affects neutrophil activity. Specifically, the aim of this study was to evaluate the cholesterol content of CF neutrophil membranes and to examine the impact of altered cholesterol content on CF membrane lipid raft structure.

Neutrophils were isolated from stable patients with CF homozygous for the ΔF508 mutation (n=6), healthy controls (HC) (n=6), or non-CF bronchiectasis patients (inflammatory control (n=6)). Neutrophil membranes and lipid rafts were isolated by subcellular fractionation and density gradient ultracentrifugation. Cholesterol levels were quantified by a fluorometric assay. Key lipid raft proteins were examined by Western blot analysis. Ethical approval was obtained from Beaumont Hospital Ethics Committee.

Proteomic and Western blot analysis confirmed reduced levels of integral scaffolding lipid raft proteins flotillin-1 and flotillin-2 expression in CF plasma membranes and lipid rafts compared to non-CF bronchiectasis and HC samples (n=7, *P<0.05, **P<0.01). Neutrophil plasma membranes and isolated lipid rafts from CF individuals had significantly decreased cholesterol content when compared to HC samples (n=6, *P<0.05, **P<0.01).

The outcome of this study demonstrates an intrinsic defect in neutrophils of individuals with CF. We have shown that reduced cholesterol content of CF neutrophil membranes results in lipid raft instability. This instability is further demonstrated by a reduction in levels of flotillin-1 and flotillin-2 in CF lipid rafts. Collectively, these results may in part explain the dysregulated activity of CF neutrophils.

This study was funded by Science Foundation Ireland, grant number 11/RFP/BMT/3094.

D11

EFFECT OF P-GLYCOPROTEIN INHIBITION ON THE BRAIN DISTRIBUTION AND ANTIDEPRESSANT-LIKE ACTIVITY OF ESCITALOPRAM IN RODENTS

F.E. O'Brien[1,2,3], R.M. O'Connor[3], G. Clarke[1,4], T.G. Dinan [1,4], B.T. Griffin[2], J.F. Cryan[1,3]
[1]Alimentary Pharmabiotic Centre, [2]School of Pharmacy, [3]Department of Anatomy & Neuroscience, and [4]Department of Psychiatry, University College Cork, Ireland.

Recent pharmacogenetic studies have highlighted a potential link between the drug efflux transporter P-glycoprotein (P-gp) and response to treatment with escitalopram, one of the most commonly prescribed antidepressants (1-2). The present studies investigated the effects of P-gp inhibition on the pharmacokinetics and pharmacodynamics of escitalopram, after both acute and chronic treatment, in rodents. To investigate the translational relevance of rodent findings, *in vitro* studies were undertaken to determine interactions between escitalopram and human P-gp.

In vitro bidirectional transport studies, using MDCKII-MDR1 cells, revealed that escitalopram was a transported substrate of human P-gp (efflux ratio = 7.22; attenuated by co-incubation of a P-gp inhibitor). Intracerebral microdialysis-based acute pharmacokinetic studies in conscious freely moving rats showed that administration of the P-gp inhibitor ciclosporin (CsA) resulted in a 67±14% increase in escitalopram concentration in microdialysis samples. In addition, samples taken at termination of these studies revealed that brain tissue concentrations of escitalopram were elevated by over three-fold in CsA treated rats relative to vehicle-treated controls (21.8±1.7 µg/g *versus* 7.1±0.4 µg/g, respectively), with no difference in plasma escitalopram levels (n=5 per group). Pharmacodynamic studies demonstrated that pre-treatment with the P-gp inhibitor verapamil enhanced the antidepressant-like effect of escitalopram in the tail suspension test (TST), due to enhanced brain levels of escitalopram (n=8-17 per group). Chronic pharmacokinetic studies revealed that, after co-administration of verapamil and escitalopram for 22 days, brain tissue levels of escitalopram were increased by over two-fold in mice treated with verapamil relative to saline-treated controls (78±20 ng/g versus 36±11 ng/g, respectively).

Pharmacokinetic studies reveal that pharmacological inhibition of P-gp results in enhanced brain levels of escitalopram in rodents, after both acute and chronic treatment, thereby demonstrating that P-gp plays a key role in the BBB transport of escitalopram. Moreover, behavioural studies show that the antidepressant-like activity of escitalopram can be augmented by P-gp inhibition *in vivo*. In addition, *in vitro* studies confirmed that escitalopram is a transported substrate of human P-gp, thereby indicating that findings in rodents may translate to humans.

Taken together, these data suggest that adjunctive therapy with a P-gp inhibitor may represent a promising approach to augment antidepressant response, or facilitate reduced antidepressant dose to limit peripheral side-effects while maintaining response, in the future.

References

[1] Lin KM *et al.* (2011). *Pharmacogenet Genomics* **21:** 163-170
[2] Singh AB *et al.* (2012). *Transl Psychiatry* **2:** e198.

C1

CORRECTION >80% OF CF CAUSING MUTATIONS IN CF CELLS USING ZFN HOMOLOGY-DIRECTED REPAIR

J. A. Hollywood[1,2], C. M. Lee[1,2] , K. Kaschig[1,2] , R. Flynn[1], M.F.Scallan[2], P.T.Harrison[1]
[1]Department of Physiology & BioSciences Institute and [2]Department of Microbiology, University College Cork, Cork, Ireland.

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene which encodes a cAMP gated anion channel expressed on the apical membrane of secretory epithelial cells. The most common mutation in *CFTR* is a CTT deletion (ΔF508) which severely disrupts CFTR channel activity. In 1989 it was shown that CFTR cDNA could correct Cl- efflux in cells derived from CF patients, and this triggered a search for an efficient and effective way to deliver cDNA to patients. To date, this approach has not resulted in clinical benefit. An alternative to cDNA addition, which can only transiently restore activity, is to permanently correct the gene defect using zinc finger nuclease (ZFN) homology-directed repair (HDR).

Gene correction requires the introduction of a donor DNA molecule containing the correct sequence to cells which then triggers HDR of the mutant gene. The use of restriction enzymes ZFNs to create a targeted double stranded break (DSBs) in the DNA can increase the rate of HDR.

We recently published the first description of correction of the ΔF508 mutation in a tracheal epithelial cell line by HDR using ZFNs and a donor plasmid[1]. At present the approach is limited to correcting one mutation. To address this, we are using our existing cftr-specific ZFNs to target a cftr mini-gene to intron 9 to correct CF mutations throughout exons 10-24 (~80% of all CF mutations) with a single ZFN pair/donor plasmid. As proof-of-principle that our ZFNs can target exogenous sequences to intron 9 we precisely incorporated a Tag sequence into the cftr gene at the ZFN target site by HR. Analysis of cells treated with ZFNs and a 1.5kb donor plasmid by nested PCR revealed that the Tag had been successfully introduced into the cftr gene at the correct location.

To effect gene repair, we have constructed a mini-gene repair construct with appropriate slice acceptor and poly- A sites, wild type exons 10-24, a T2A linker and GFP tag (to allow co-production of a GFP protein along with the functional CFTR protein and also for fluorescence imaging, to identify cells which have been corrected), flanked by 750 bp homology arms. Incorporation of the mini-gene would result in full length corrected CFTR mRNA production that's under the control of the endogenous promoter, a major limitation experienced when delivering exogenous cDNA. This strategy has previously been used to replace exons 2-8 of the F9 gene to restore haemostasis in haemophilic mice[2]. Successful gene correction using a CFTR mini-gene would result in normal spatiotemporal expression of the corrected CFTR gene, is permanent for the lifetime of the cell and is not subject to gene silencing. It could be of use as an alternative strategy to cDNA addition for gene therapy.

References

1. Lee CM, Flynn R, Hollywood JA, Scallan MF, Harrison PT. Correction of the ΔF508 Mutation in the Cystic Fibrosis Transmembrane Conductance Regulator Gene by Zinc-Finger Nuclease Homology-Directed Repair.

Bioresearch Open Access. 2012 Jun;1(3):99-108. doi: 10.1089/biores.2012.0218.

2. Li H, Haurigot V, Doyon Y, Li T, Wong SY, Bhagwat AS, Malani N, Anguela XM, Sharma R, Ivanciu L, Murphy SL, Finn JD, Khazi FR, Zhou S, Paschon DE, Rebar EJ, Bushman FD, Gregory PD, Holmes MC, High KA. In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature. 2011 Jun 26;475(7355):217-21. doi: 10.1038/nature10177.

C2

APATHY & PARKINSON'S DISEASE: A HIGH ANGULAR RESOLUTION DIFFUSION IMAGING STUDY

W. Quinn[1,2], T. Vidal[3], I. Rieu[3], M. Perie[3] , F. Durif[2,3].
[1]Cork Institute of Technology, Cork, Ireland, [2]Université d'Auvergne, Clermont-Ferrand, France, and [3]Hôpital Gabriel Montpied, Clermont-Ferrand, France.

Parkinson's disease (PD) is mainly associated with its motor symptoms but it also displays non-motor symptoms including neuropsychiatric and cognitive dysfunction. One of the most poorly recognised aspects of PD is apathy which manifests itself as a bluntness of emotion and lack of motivation. A number of studies have suggested that apathy, which occurs in one third to one half of PD cases, may be a symptom/syndrome which is separate from depression and that mis-diagnosis can be detrimental to the effectiveness to rehabilitation programs. It has been hypothesised that apathy occurs due to disruption of the mesocorticolimbic pathway, also known as the reward pathway, through the loss of dopaminergic neurons in the Ventral Tegmental Area (VTA)[1].

In this project Diffusion Weighted (DW) Imaging is used to examine the effects of PD on the fibres of the mesocorticolimbic pathway and the correlation between these effects and apathy. DW imaging is a modality in Magnetic Resonance Imaging (MRI) which can provide in-vivo information regarding the diffusion properties of white matter fibre bundles. The measured macroscopic diffusion properties are believed to be related to the microscopic properties of the underlying axons and can provide important information regarding the condition of these axon bundles[2]. In this study a High Angular Resolution Diffusion Imaging (HARDI) protocol is used examine the diffusion properties of reconstructed diffusion streamlines which project from the VTA to the constituent structures of the mesocorticolimbic system. In order to investigate the effects of apathy on the mesocorticolimbic pathway three cohorts matched for age, sex, and disease duration have undergone both DW-MRI and MR anatomic scans. Each cohort comprises of twenty subjects with cohorts classified according to a neuropsychological assessment as; subjects with PD which display apathy, subjects with PD who do not display apathy and healthy controls. The diffusion parameters of each cohort is compared statistically in order to determine if there is a correlation between the measured diffusion parameters of each cohort and the occurrence of apathy.

A method of identifying apathy in PD is essential especially when apathy and depression occur simultaneously. It is believed that from this study a greater understanding of apathy in PD will be gained which will eventually lead to more

effective rehabilitation and treatment procedures. This presentation will describe the process involved in extracting the required data and preliminary results of the study.

Ethical committee approval was obtained for this study.

References

1. Kirsch-Darrow L, Marsiske M, Okun MS, Bauer R, Bowers D. Apathy and depression: separate factors in Parkinson's disease. J Int Neuropsychol Soc., 2011, Vol. 17, pp 1058-66.

2. Beaulieu C. *The* basis of anisotropic water diffusion in the nervous system - a technical review. NMR in biomedicine, 2002, Vol. 15, pp 435-455.

C3

SENSOR DEVICES FOR HEALTH APPLICATIONS BASED ON SMART MINIATURISED MICROSYSTEM TECHNOLOGY

E. Moore, W. Messina, U. Crowley, G. Duffy, M. Fitzgerald
Life Science Interface, Tyndall National Institute, University College Cork, Cork, Ireland.

The component and systems business in Europe concentrates on added value operations, on system integration, on novel technologies and on enabling the end-user industry to offer new technologies and total product/service solutions. Increased multi-disciplinary, integrated software/hardware systems, heterogeneous microsystems and the use of widely distributed systems for monitoring control are growing challenges. Microsystems exist to integrate and interface multiple core technologies and related materials to implement a variety of functions. They are implemented through scalable homogeneous or heterogeneous hardware integration technologies in order to advance miniaturisation, functionality and reliability of the sensing, processing, actuating and communication functions. This paper presents a range of sensing (electrochemical, impedance, immunosensor etc.) and separation devices (capillary electrophoresis) that are being developed at Tyndall National Institute which, are module in nature and can be integrated into lab-on-chip systems. We are using tools and processes of nano/micro-technology to address challenges in biology with the objective to develop miniaturised devices and systems that are simple, label-free, and sensitive, and developed according to end user requirements. These integrated systems will address markets including health, pharmaceutical industry, environmental monitoring, food, beverage and security/defence sectors.

C4

A NOVEL PLATELET FUNCTION TEST RAPIDLY DETECTS DRUG EFFECTS IN PATIENTS WITH CARDIOVASCULAR DISEASE

K. Egan[1,2], A. Lopez Alonso[1,2], B. Jose[2], N. Gilmartin[2], P. McCluskey[2], B. Byrne[2], A. Ricco [2,3], D. Kenny [1,2].

[1] Royal College of Surgeons in Ireland, Dublin, Ireland, [2] Biomedical Diagnostics Institute, Dublin City University, Ireland, and [3] NASA Ames Research Center, California, USA.

Antiplatelet agents are used to prevent major events such as myocardial infarction in patients with cardiovascular disease (CVD). Despite their proven benefit, major events still occur in CVD patients taking antiplatelet agents. It has become apparent that high numbers of patients show a poor response to therapy, (i.e.) incomplete platelet inhibition. This has been linked to an increased incidence of major events in CVD patients. Consequently, a rapid, accurate assay of antiplatelet drug effect is needed.

We have developed a simple, whole blood based, platelet adhesion assay capable of accurately measuring the effect of antiplatelet agents. In the assay, unactivated platelets adhere to 6 µm fibrinogen spots. The addition of platelet agonists decreases platelet adhesion to fibrinogen spots, by promoting platelet activation and aggregation. However, in the presence of an agonist specific inhibitor, this decrease in platelet adhesion does not occur. Hence, the assay measures the inability of agonists to decrease adhesion in the presence of antiplatelet agents. For example, in healthy volunteers, the basal level of platelet adhesion is 75±8% (mean ± SD, n=6). The platelet agonist arachidonic acid (AA, 0.5 mM) causes a significant decrease in platelet adhesion (18±6 %, n=6, $p<0.05$, paired t test). Aspirin (50 µM, inhibits COX-1 dependent generation of the platelet agonist Thromboxane A_2 from AA) inhibit this AA induced decrease in platelet adhesion (66±8 %, n=6), demonstrating the ability of the assay to detect antiplatelet effects *in vitro*. In CVD patients taking aspirin, platelet adhesion in untreated and AA treated blood samples are comparable (78±11% vs. 65 ±13%, n=17) demonstrating the ability of the assay to detect antiplatelet effects *ex vivo*. The assay has also been optimised to detect the effect of all other clinically relevant antiplatelet agents, including P2Y12 and αIIbβ3 receptor antagonists. In comparison with other platelet function tests, the assay shows an excellent correlation with the results of flow cytometry (Pearson Correlation Coefficient = - 0.9 vs. αIIbβ3 activation, $p < 0.001$), a highly sensitive, but complex method of testing platelet function, a fact that limits its use in a clinical scenario.

In conclusion, we have developed a simple, sensitive, whole blood based platelet function test that can accurately detect the effect of antiplatelet therapies *in vitro* and *ex vivo*. Due to its simplicity and sensitivity, the assay has potential for widespread use in the monitoring and tailoring of antiplatelet therapies in CVD patients.

C5

AN ANIMAL MODEL FOR THE INVESTIGATION OF THE MECHANISM OF ACTION OF SACRAL NEUROMODULATION

J. Evers [1], E.V. Carrington[1,2], S.M. Scott SM, C.H. Knowles[3], P.R. O'Connell [1,4], J.F.X. Jones[1].

[1]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, [2]GI Physiology Unit, The Wingate Institute of Neurogastroenterology, Queen Mary, University of London, London, United Kingdom, [3]National Centre for Bowel Research and Surgical Innovation, Queen Mary, University of London, London, United Kingdom, [4]Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland.

Sacral neuromodulation (SNM) is now routinely used in the treatment of faecal incontinence, although its mechanism of action is still unclear. Our hypothesis is that SNM may augment cortical sensory processing of anal canal receptor mediated inputs. Therefore, anal canal cortical potentials (CPs) evoked either electrically or mechanically were studied before and after SNM.

Experimental protocols were approved by the UCD Animal Ethics Research Committee and licenced by the Irish Department of Health and Children. In 14 female virgin Wistar rats (body mass: 200g–300g) a craniotomy was performed over the right primary somatosensory cortex under urethane anaesthesia (1.5 $g.kg^{-1}$, i.p.). An extra dural 32-channel multi-electrode array, was used to record CPs evoked by electrical anal canal, left tibial nerve and median nerve stimulation (n=6) or mechanical hind-paw, fore-paw and anal canal stimulation (n=8). Electrical stimulation was achieved with a 2mm gold plug cathode and mechanical stimulation with an interdental brush mounted on a stepper motor. SNM (10Hz, 15V, 1ms pulse duration, 3min) was applied via a concentric electrode placed in the left first sacral foramen. Post SNM CPs are expressed as percentages of initial value (mean ± s.e.m.); the criterion for statistical significance was $P<0.05$.

All evoked CPs were similar for electrical and mechanical stimulation. SNM induced an increase in the maximal amplitude in anal (49.8%±10.91% (electrical) and 35.7%±16.15% (mechanical) and hind-paw (61.4%±1.22%/88.0%±44.33%), but not in fore-paw CPs (-10.2%±4.24%/-7.66%±7.67%). Two-tailed t-tests with subsequent Bonferroni correction for multiple comparisons confirmed that SNM causes statistically significant potentiation in anal and hind-paw CPs, but not in fore-paw CPs.

SNM potentiates afferent fibre input to shared spinal segments but not higher segments. This study provides evidence that SNM selectively restores sacral inputs and may improve cortical awareness of the anorectum. The finding that a lower limb nerve input is also augmented may serve as a surrogate marker of the efficacy of SNM. This finding also suggests that percutaneous posterior tibial nerve stimulation, a novel treatment for incontinence, may operate similarly to SNM.

The authors acknowledge the support of Science Foundation Ireland.

C6

LIPOXIN A_4 REDUCES cAMP DEPENDENT ION TRANSPORT IN T84 CELLS

M.I. Hollenhorst[1,2], V. Urbach[1].

[1]National Children's Research Centre, Dublin, Ireland; [2]Royal College of Surgeons, Dublin, Ireland.

Lipoxin A_4 (LXA_4) is an endogenous lipid mediator that is well known for its beneficial properties in resolving inflammation. Mostly LXA_4 displays its actions by binding to the G-protein coupled formyl peptide receptor 2 (FPR2). LXA_4 analogues have been shown to attenuate the inflammation in inflammatory bowel diseases such as colitis. Often, these diseases are also characterised by defective ion transport. However, the impact of LXA_4 on gastrointestinal tract epithelial ion transport is still unknown. Yet, it was reported that LXA_4 regulates airway epithelial ion transport[1]. Thus, this study investigates the putative influence of LXA_4 on intestinal epithelial ion transport.

To examine the impact of LXA_4 on transepithelial ion transport colonic T84 cells were grown in monolayers on filters and transepithelial ion current was recorded in Ussing chambers. Additionally expression of FPR2 protein in the human colonic cell lines HT29Cl.19A and T84 was investigated by western blot, FACS (fluorescence activated cell sorting) and immunofluorescence analysis.

We were able to detect the presence of the LXA_4 receptor FPR2 in HT29Cl.19A cells by western blot and FACS experiments. Similarly, FPR2 protein expression was detected in T84 cells by western blot, FACS and immunofluorescence analysis. Application of LXA_4 apically (1 nM, 10 nM or 100 nM) did not influence baseline transepithelial ion current in T84 cells compared to control conditions in any of the tested concentrations. Additionally there was no significant influence of LXA_4 (10 nM, apical) on the ion current increase induced by the calcium ionophore A23187 (2 µM, apical). Thus, LXA_4 did not influence calcium-dependent ion current. However, presence of 100 nM LXA_4 apically significantly reduced the current increase that was evoked by application of 10 µM forskolin apically.

Thus, LXA_4 inhibits cAMP-dependent ion current in T84 cells. This might be beneficial for reducing the excess colonic fluid secretion observed in colitis by reduction of ion secretion that also regulates water secretion into the colon.

The authors acknowledge grant support from the Health Research Board of Ireland and the National Children's Research Centre and the French National Institute of Health (*INSERM)*.

Reference

1. Verrière V., Higgins G., Al-Alawi M., Costello R.W., McNally P., Chiron R., Harvey B.J., Urbach V. Lipoxin A4 stimulates calcium-activated chloride currents and increases airway surface liquid height in normal and cystic fibrosis airway epithelia. PLoS One, 2012; 7(5):e37746.

C7

NOVEL ROLE OF PREGNANCY SPECIFIC GLYCOPROTEINS IN PLATELET INTEGRIN BINDING

K. Golla[3], D.K. Shanley[1], P.A. Kiely[2], S. Allen[3], R.T. O'Riordan[1], N. Moran[3], T. Moore[1].
[1]Department of Biochemistry, University College Cork, Cork, Ireland, [2]Department of Life Sciences, and Materials and Surface Science Institute, University of Limerick, Limerick, Ireland, and [3]Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland.

Pregnancy-specific glycoproteins (PSGs) are immunoglobulin superfamily members encoded by multigene families in rodents and primates. In human pregnancy, PSGs are secreted by placental syncytiotrophoblast, and reach concentrations of up to 400µg/ml in the maternal bloodstream at term. RGD tri-peptide motifs, a known integrin-binding motif, are identified in the majority of human PSGs suggesting that they may function as integrin ligands or inhibitor ligands. We noted that human PSG1 has a KGD, rather than an RGD motif. The KGD sequence is present in barbourin snake venom and has the ability to bind to platelet integrin αIIbβ3[1].

Here we show that human platelets bind to recombinant human PSG1 proteins. PSG1 binds and inhibits the platelet – fibrinogen interaction in a dose-dependent manner. However, the KGD is not crucial for the interaction with platelet integrin αIIbβ3 suggesting that PSG1 can bind to multiple regions of αIIbβ3.

Our results suggest that during pregnancy the PSG competes with fibrinogen in binding to αIIbβ3 preventing platelet aggregation and thrombosis in the prothrombotic maternal environment of pregnancy.

Reference

1. Scarborough RM, Rose JW, Hsu MA, Phillips DR, Fried VA, Campbell AM, Nannizzi L, Charo IF. Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of Sistrurus m. barbouri**.** J Biol Chem. 1991; 266(15):9359-62.

C8

LIPOXIN STIMULATED EPITHELIAL REPAIR IS MEDIATED THROUGH POTASSIUM CURRENTS

P.J. Buchanan[1,2], P. McNally[1], V. Urbach[1,2,3].

[1]National Children's Research Centre, Our Lady's Children Hospital, Dublin 12, Ireland, [2]Department of Molecular Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland, and [3]INSERM U845, Faculté de Médecine, Paris, France.

Progressive lung destruction is the main cause of morbidity and mortality in cystic fibrosis (CF) due to persistent bacterial infection and inflammation along with a reduced capacity for epithelial repair. Levels of the anti-inflammatory mediator Lipoxin A_4(LXA_4) have been reported to be reduced compared to inflammatory mediators in bronchoalveolar lavages of patients with CF. We previously shown that LXA_4 targets airway epithelial cells and stimulates tight junction formation[1]. We investigated the ability of LXA_4 to trigger epithelial repair through the initiation of proliferation and migration in non-CF(NuLi-1) and CF(CuFi-1) human airway epithelia.

Spontaneous epithelial repair (n=8, $p<0.001$) and cell migration (n=7, $p<0.001$) using a scratch assay and boyden chamber assay was determined to be significantly slower in CF epithelial cultures (CuFi-1) compared to controls (NuLi-1). LXA_4 triggered an increase in cell migration (n=6,$p<0.001$), proliferation (n=6, $p<0.05$) (MTT assay) and wound repair (n=6,$p<0.005$) in non-CF and cystic fibrosis airway epithelia. These responses to LXA_4 were completely abolished by the FPR2 (LXA_4receptor) antagonist, Boc2. The K_{ATP} channel opener, pinacidil, mimicked the LXA_4 effect significantly increasing migration (n=6,$p<0.001$), proliferation (n=6, $p<0.01$) and epithelial repair (n=6, $p<0.001$) while the K_{ATP} channel inhibitor, glybenclamide, blocked the responses to LXA_4. LXA_4 did not affect potassium channel expression (n=4)(PCR) but significantly up regulated glybenclamide-sensitive(K_{ATP}) currents through the basolateral membrane (Ussing chamber) of NuLi-1 (n=4,$p<0.01$) and CuFi-1 (n=4,$P<0.05$) cells. The MAP kinase (ERK1/2) inhibitor, PD98059, also inhibited the LXA_4 induced proliferation of NuLi-1 and CuFi-1 cells. Finally, both LXA_4 and pinacidil stimulated ERK-MAP kinase phosphorylation (n=4,$p<0.01$) (Western Blot), while the effect of LXA_4 on ERK phosphorylation was inhibited by Glybenclamide.

This work highlights a potential therapeutic for exogenous delivery of LXA_4 to restore levels in CF patient's and thus reduce lung destruction.

The authors acknowledge grant support from Health Research Board of Ireland, The National Children Research centre and the French National Institute of Health (INSERM).

Reference

1. Grumbach, Y., Quynh, N.V., Chiron, R., Urbach, V. LXA_4 stimulates ZO-1 expression and transepithelial electrical resistance in human airway epithelial (16HBE14o-) cells. Am J Physiol Lung Cell Mol Physiol., 2009; 1: 101-108.

B1

VISCERAL PAIN SENSITIVITY AND STRESS-INDUCED DEFECATION IN THE WISTAR KYOTO RAT MODEL OF IBS IS SUPPRESSED BY INHIBITION OF INTERLEUKIN-6 SIGNALLING

M.M. Buckley[1,2] , D. O'Malley[2].
[1]Alimentary Pharmabiotic Centre and [2]Department of Physiology, University College Cork, Cork, Ireland.

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by episodic bouts of altered bowel habit, bloating and abdominal pain, but the underlying pathophysiology is still poorly understood. There is an increase in circulating levels of the proinflammatory cytokine interleukin-6 (IL-6) in IBS patients compared with control patients and as a consequence, IL-6 has been proposed as a biomarker of the disorder. Furthermore, a relationship has been demonstrated between activation of central corticotropin-releasing factor $(CRF)_1$ receptors and the induction of stress-related IBS symptoms. Crosstalk between IL-6 and CRF has also been postulated. The aim was to investigate the role of IL-6 mediated signalling in visceral pain sensitivity and stress-induced defecation.

Adult male Sprague Dawley (SD, n=12 per group) and stress-sensitive Wistar Kyoto (WKY, n=12 per group) rats were treated either with anti-IgG (1mg/kg Day 0), xIgG and anti-IL-6 receptor (xIL-6R, 1mg/kg, day 0 and 0.5mg/kg days 3 and 10) or xIgG, xIL-6R and the CRFR1 antagonist, antalarmin (10mg/kg, 1 hour prior to the behavioural stressor). The first behavioural test on day 6 measured stress-induced faecal output in an open field arena (10 minute trial). Rats were assessed for changes in visceral hypersensitivity in response to colorectal distension (ramp 0-80mmHg, 8 minutes) on day 13.

The number of pellets excreted by the stress-sensitive WKY rats treated with IgG was higher than SD controls consistent with control data in these rat strains. Treatment with xIL-6R antibodies caused a decrease in faecal output in WKY rats in the open field trial ($P<0.01$, n=12). This was further reduced by co-administration of antalarmin ($P<0.001$, n=12). The threshold at which pain behaviours in response to colorectal distension were observed was increased following administration of xIL-6R ($P<0.001$, n=9) and this effect was further potentiated by co-application of antalarmin ($p<0.001$, n=12).

These *in vivo* studies demonstrate for the first time that blocking peripheral IL-6 signalling via IL-6Rs has functional outcomes. Two key pathophysiological symptoms of the WKY model of IBS, stress-induced faecal output and visceral hypersensitivity, were alleviated by neutralising IL-6Rs and this effect was enhanced in the presence of the CRFR1 antagonist, antalarmin. These novel findings reveal a potential therapeutic target for the treatment of IBS symptoms.

B2

PLATELETS ENHANCE INVASION OF OVARIAN CANCER CELLS IN AN EXPERIMENTAL METASTASIS MODEL

N.M. Cooke[1,3], K. Egan[1,3], C. Spillane[2,3], J. O'Leary[2,3], D. Kenny[1,3].
[1]Department of Molecular and Cellular Therapeutics, RCSI, Dublin 2, [2]Department of Histopathology, Trinity College Dublin, and [3]The Biomedical Diagnostics Institute, Dublin City University, Dublin, Ireland.

Platelets have a critical but incompletely understood role in cancer. Mortality from most cancers is related to secondary spread of the tumour. It has been shown that platelets interact with cancer cells in the bloodstream. Ovarian cancer is a lethal malignancy since over 70% of ovarian cancer patients present with advanced metastatic disease. It is unclear why different ovarian cancers have different metastatic phenotypes. Our hypothesis is that platelet interactions with ovarian cancer cells alter the metastatic potential of these cells. We used an experimental model of metastasis using matrigel chambers to investigate this hypothesis.

Two metastatic ovarian cancer cell lines, 59M and SKOV3, were studied. We have previously shown that the ability of these cancer cell lines to activate platelets *in vitro* is significantly different from one another. To further interrogate this finding, the invasive capacity of these cancer cells alone, cloaked with platelets, and in the presence of platelets pretreated with an antiplatelet agent (20μM, aspirin) was investigated. Cancer cells alone were compared to those incubated with platelets or with aspirin-treated platelets. Cell suspensions were added to matrigel chambers in triplicate, and allowed to invade for 16 and 24 hours in a humidified atmosphere containing 5% CO_2 at 37°C. Non-invading cells were removed. Cells on the lower surface of the membrane were fluorescently labelled and examined by an epifluorescence microscope.

The invasion of SKOV3 cells incubated with platelets was significant greater after 16 and 24 hours (n=6, $P < 0.0283$ and $P = 0.0001$, respectively) than SKOV3 cells alone. In contrast, platelets did not enhance invasion of the already aggressive 59M cells. Invasion, as measured by the matrigel assay, was the same between 59M cells alone and SKOV3 cells cloaked with platelets (n=6, 48% versus 46% at 16 hours, respectively, and both at 42% at 24 hours). Since aspirin has a role in the prevention of cancer and is a potent antiplatelet agent, we pretreated platelets with aspirin (30 min at 37°C). Aspirin abrogated the platelet-mediated enhancement of invasion in SKOV3 cells.

These results demonstrate that platelets enhance the metastatic potential of ovarian cancer cells. They also demonstrate for the first time that the antiplatelet agent aspirin attenuates this effect.

The authors acknowledge grant support from Science Foundation Ireland as part of the Biomedical Diagnostics Institute for Science Excellence and Technology (10/CE/B1821).

B3

HIGHLY EFFICIENT NON-VIRAL GENE-ACTIVATED MATRICES INCORPORATING ANGIOGENIC AND OSTEOGENIC GENES ENHANCES BONE TISSUE REGENERATION IN VIVO

E.G. Tierney[1,2], C.M. Curtin[1, 2], S.A. Cryan[3], G.P. Duffy[1,2], F.J. O'Brien[1, 2].
[1]Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, [2]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland, and [3]School of Pharmacy, Royal College of Surgeons in Ireland, Dublin, Ireland.

Treatments combining nanotechnology with gene and stem cell-based therapies on biodegradable extracellular matrices are increasingly showing potential in bone tissue engineering. Similarly, gene-activated matrices (GAMs) have shown potential in localised gene delivery resulting in bone tissue regeneration.

In this study, the ability of polyethyleneimine (PEI) and nano-hydroxyapatite (nHA) particles, developed in-house, to act as non-viral vectors for delivery of plasmid-DNA when combined with our collagen-nHA (coll-nHA) scaffolds specifically tailored for bone repair, yielding gene-activated matrices (GAMs) [1, 2], was determined. In addition, coll-nHA-dual gene scaffolds (dual GAMs) containing both an angiogenic gene, VEGF, and an osteogenic gene, BMP2, were assessed for bone healing in an *in vivo* Wistar rat calvarial defect model.

When cells were applied to the coll-nHA scaffolds under osteogenic conditions *in vitro*, the dual GAMs exhibited significantly superior osteogenic potential when analysed using microCT, calcium quantification and histology compared to single-gene GAMs and non-transfected cell controls. When the dual GAMs were assessed *in vivo*, the nHA dual GAM outperformed all other groups as early as 4 weeks post-implantation as determined using X-ray, microCT, quantification of new bone volume, histology and vessel formation. One-way ANOVA followed by a Holm-Sidak test was carried out and $p<0.05$ was considered to indicate statistical significance.

This research has demonstrated the potential of using novel coll-nHA scaffolds as GAMs for therapeutic gene therapy while also being capable of simultaneously delivering numerous genes. This study underlines the effect of specifically tailoring GAMs for bone regeneration applications and furthermore, this novel delivery system may be used for the regeneration of numerous other tissues in addition to bone.

The authors acknowledge the European Research Council (ERC grant agreement n°. 239685) and Science Foundation Ireland, President of Ireland Young Researcher Award (04/Yl1/B531).

References

1. Tierney, E.G. *et al*. The development of non-viral gene-activated matrices for bone regeneration using polyethyleneimine (PEI) and collagen-based scaffolds. J Control. Release, 2012; 158(2):304-11.
2. Curtin, C.M. *et al*. Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation Adv. Mat. 2012, 24(6):749-54.

P1

HAWTHORN JUICE AND BLUEBERRY JUICE INCREASE HYDROGEN SULFIDE PRODUCTION IN RAT KIDNEY *IN VITRO*

M. Hynes*, A. L. Ní Churraoin*, L. A. Horrigan
Physiology, School of Medicine, National University of Ireland, Galway, Ireland.
* Equal contributors to the study

Hawthorn and blueberry are dietary agents often used for their cardiovascular and other health benefits, but their mechanisms of action are largely unknown. Hydrogen sulfide (H_2S) has recently become recognised as an important regulator of many physiological functions. Given some evidence that blueberry juice may mediate vasorelaxation via H_2S production[(1)], this study investigated the effect of blueberry juice, hawthorn juice and aged garlic extract on H_2S production in rat kidney *in vitro*.

Male Sprague-Dawley rats were used in accordance with the NUI Galway Animal Care and Research Ethics Committee procedures. Following animal sacrifice, the kidneys were removed and homogenised (1/10 w/v) in a pH 7.4 phosphate buffer. The pH of dietary agents was also adjusted to 7.4. Samples were then incubated (15 mins; 37^{o}C) with buffer (control), hawthorn juice, blueberry juice or aged garlic extract at dilutions of 1:5 (agent:homogenate). Following incubation, samples and standards (NaHS) were assayed by the methylene blue method for sulfide detection. Samples were assayed both with and without L-cysteine (10mM), the precursor for H_2S production. Data was analysed by One-Way ANOVA followed by Dunnett's post hoc test, and expressed as mean $\pm$ s.e.m. with n = 4 – 6 for each treatment.

In the absence of L-cysteine, hawthorn juice caused a significant increase in sulfide compared to control (p = 0.001), with 3.13 $\pm$ 0.71 ・・moles/g tissue detected, compared to 0.5 $\pm$ 0.5 ・moles/g tissue in control. In the presence of L-cysteine, both hawthorn juice (p < 0.001) and blueberry juice (p < 0.001) caused significant increases in sulfide compared to control, with 19.01 $\pm$ 2.30 ・moles/g tissue and 13.09 + 1.02 ・moles/g tissue produced respectively, compared to 1.01 $\pm$ 0.36 ・moles/g tissue detected in control. Aged garlic extract had no significant effect.

It has not previously been demonstrated that agents rich in polyphenols effect H_2S production. This is of great interest as it may at least partly explain the mechanism of action of these dietary agents.

Reference

1. Horrigan LA, Holohan CA, Lawless GA, Murtagh MA, Williams CT, Webster CM. Blueberry juice causes potent relaxation of rat aortic rings via the activation of potassium channels and the H(2)S pathway. Food Funct. 2013 Feb 26;4(3):392-400.

P2

Lipoxin A_4 Delays the Invasion of Cystic Fibrosis Bronchial Epithelial Cells by the pathogen *Pseudomonas aeruginosa*

G. Higgins[1,2] , P. McNally[1], V. Urbach[1,2]

[1]National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland, and [2]Royal College of Surgeons in Ireland, Dublin, Ireland.

P3

A COMPARISON OF BEHAVIOURAL PROFILES BETWEEN COMMERCIALLY OBTAINED AND IN-HOUSE BRED RATS.

K. Bannerton, K. McCabe, Z. McAleavey, J.P. Kelly.

Discipline of Pharmacology and Therapeutics, School of Medicine, NUI Galway, Ireland.

Preclinical behavioural pharmacological evaluation is a pivotal element in the development of drugs to treat a range of psychiatric and neurodegenerative disorders. As such, it is imperative that such tests are performed in a consistent manner, to produce reliable, reproducible results of both baseline and drug-induced behaviours. However, examination of the literature suggests that results of these tests can vary immensely, with variation being accredited to strain and age of animals used. One variable that has recently been implicated in such variation is the breeding supplier of rats of the same strain[1]. Whether these effects are attributable to genetic differences or differences in early life rearing of animals has not been established. Thus, the aim of this study was to compare the baseline behavioural profiles between male, Sprague-Dawley rats (8-11 weeks at testing), from the same genetic background, differing only in whether they were bred in-house (IHB) or commercially bred (CB) and obtained from the same commercial supplier as the parents of the offspring.

The behaviour of these rats was investigated in some of the most commonly employed behavioural tests. Independent *t*-test or Mann-Whitney U test showed that for all tests, there were no significant differences in baseline behaviours between the IHB and CB rats ($p>0.05$ for all): the elevated plus maze (percentage open arm entries (%OAE):IHB, 46±6 vs. CB, 41±9; percentage open arm time (%OAT):IHB, 38±9 vs. CB, 32±12), the open field (Distance Moved: IHB, 2597±341 vs. CB, 2503±515), the light/dark box (Percentage time in light box: IHB, 8±17 vs. CB, 12±12), and the resident-intruder test (Social Interaction: IHB, 178±54 vs. CB, 160±24; Aggression: IHB, 102±22 vs. CB, 62±50). The depressive-like behaviour of these animals was assessed in the forced swim test (Immobility: IHB, 219±44 vs. CB, 199±29; Climbing: IHB, 66±48 vs. CB, 83±40; Swimming: IHB, 15±15 vs. CB, 19±17), as well as the rats' learning and memory abilities in the Morris water maze test (Time spent in south west quadrant: IHB, 40±12 vs. CB, 43±11). All results represent mean±SD for $n=12$ animals, except time in light box, represented as median±interquartile range.

Thus, we can conclude that differences reported between suppliers may be as a consequence of the emergence of substrains, rather than due to differences in early life rearing experiences. It is intended to follow up these studies by examining whether there are differences in drug-induced behavioural responses between these two cohorts.

Reference

1. Honndorf, S., Lindemann, C., Tollner, K., Gernert, M. Female Wistar rats obtained from different breeders vary in anxiety-like behaviour and epileptogenesis. Epilepsy Research. 2011; 94:26-38.

P4

THE ROLE OF DNA METHYLATION IN TOLL-LIKE RECEPTOR 3 EXPRESSION

O'Connell, S, McKernan, D.P .
Pharmacology and Therapeutics, NUI Galway, Ireland.

Toll-like receptors (TLRs) are pattern recognition receptors integral to the innate immune response. Upon recognition of a Pathogen Associated Molecular Pattern (e.g. TLR3 can detect dsRNA), activated TLRs induce downstream signalling cascades, which culminate in the production of a variety of cytokines, which orchestrate the inflammatory response. TLRs are expressed on a wide variety of cells, including immune and non-immune cells and thus play a role in a variety of biological activities. The expression of these receptors varies with cell type and with disease progression (1). DNA methylation is an epigenetic mechanism, which the cell employs to regulate gene expression. A family of enzymes known as DNA methyltransferases (DNMT) catalyses the addition of a methyl group from *S*-adenosylmethionine to the 5′ position of cytosine in the context of CG dinucleotides (2). It is not known if epigenetic mechanisms such as DNA methylation play a role in the regulation of TLR expression. In this study, we investigated if TLRs are epigenetically regulated in the intestinal epithelial cell line, HCT116.

We examined the basal mRNA expression, by QRT-PCR, of TLRs 1-10 using intestinal epithelial wild-type (WT) and a double knockout HCT116 cell line (DKO) where both DNMT 1 and DNMT 3b genes are knocked out by homologous recombination (3). Relative mRNA expression was quantified with an Applied Biosystems AB7500 and analysis performed using the 2(-Delta Delta C(T)) Method. Statistical analysis involved using unpaired student's t-tests and Analysis of Variance (ANOVA) where appropriate. $p < 0.05$ was considered statistically significant.

Notably, basal TLR3 mRNA expression was significantly reduced in HCT116 DKO cells (WT, 0.001258 ± 0.0001612, n=9 vs. DKO, 0.000003426 ± 0.0000006183, N=9, p<0.001)Following this, we stimulated WT and DKO cells with Poly I: C (10µg/ml), a synthetic analogue of double stranded RNA, the TLR3 ligand, for 6 and 24 hours. TLR 3, IL-6, TNF-α and IFN-ß mRNA expression were measured using QRT-PCR. Cytokine mRNA expression increased following stimulation in WT cells but not in DKO cells after 6 hours. Specifically, IL-6 mRNA expression significantly increased in WT cells but not in the DKO cells. (WT 0.0001359 ± 0.00001384, n=9 vs. DKO, 0.000006379 ± 0.000001287, N=9, p<0.001) TNF-α displayed the same pattern as IL-6 (WT 0.0002819 ± 0.00001932, n=9 vs. DKO, 0.000009232 ± 0.000001295, N=9, p<0.001). IFN-ß mRNA expression also increased in WT cells exclusively. (WT 0.001492 ± 0.00006195, n=9 vs. DKO, 0.00001020 ± 0.000001196, N=9, p<0.001). TLR3 mRNA expression increased in WT cells at 24 hours. (WT UT 0.001311 ± 0.0001538, n=9 vs. WT Poly I:C, 0.007540 ± 0.001001, N=9, p<0.001).

Our findings indicate that DNA methylation is implicated in the regulation of certain TLRs. Therapeutic targeting of the epigenome might provide new opportunities for targeting the innate immune system for inflammatory disorders.

References

1. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 Feb 24;124(4):783-801. Review.
2. Bestor TH. The DNA methyltransferases of mammals. Hum Mol Genet. 2000 Oct;9(16):2395-402. Review.
3. Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 2002 4;416(6880):552-6.

P5

RETROSPECTIVE STUDY OF BONE MINERAL DENSITY IN WOMEN WITH CHRONIC HEPATITIS C INFECTION IN CORK UNIVERSITY HOSPITAL

T. Jayaraman, S. Corbett, E. Kenny-Walsh, O. Crosbie.
Department of Hepatology, Cork University Hospital, Cork, Ireland.

It is suggested that persons with chronic hepatitis C infection are at higher risk of developing osteoporosis. We aimed to study the bone mineral density and rates of osteoporosis in our cohort of women with chronic hepatitis C infection.

From the departmental database, we identified a cohort of Irish women who were exposed to Hepatitis C virus genotype 1B via contaminated anti D administered in 1977. Patients were separated into two groups: those who are chronically infected with Hepatitis C (*PCR*+) and those who had spontaneously cleared the virus as evidenced by positive antibodies (*PCR*-). Reports of the latest dual energy x-ray absorptiometry (DEXA) performed were extracted from each patient's medical records. Standard statistical methods were applied.

A total of 101 patients were identified of which 52 are *PCR*+ and 49 are *PCR*-. Mean age of *PCR*+ and *PCR*- are 63 (54-77) years and 63 (54-75) years respectively (p=0.861). Mean BMI of *PCR*+ is 27.5±4.2 kg/m^2 and *PCR*- is 26.8±5.6 kg/m^2 (p=0.495). DEXA scan measurements at the spine showed that *PCR*+ has lower BMD (1.026±0.115 g/cm^2 vs. 1.074±0.136 g/cm^2, p = 0.058), T-score (-1.29±0.97 vs. -0.88±1.13, p =0.052) and Z-score (-0.102±1.03 vs 0.439±1.01, p =0.009).Measurements at the left femur also showed that *PCR*+ has lower BMD (0.913±0.120 g/cm^2 vs. 0.934±0.135 g/cm^2, p =0.408), T-score (-0.73±1.00 vs. -0.55±1.13, p =0.412) and Z-score (0.188±0.93 vs. 0.481±0.95, p =0.126). Based on spinal T-scores, the rate of osteoporosis in *PCR*+ is 5% compared to 2% in the *PCR*- while the rate of osteopaenia in *PCR*+ and *PCR*- are 28.7% and 22.8% respectively (p=0.253). Based on left femoral T-scores, the rate of osteoporosis is 1% in both *PCR*+ and *PCR*-, while 22.2% of *PCR*+ and 16.2% of *PCR*- are osteopaenic (p=0.7).The rate of obesity is 11.9% in *PCR*+ and 10.9% in *PCR*- while 23.8% of *PCR*+ and 15.8% of *PCR*- are overweight (p=0.186).

Our results show that there is no significant difference in rates of osteoporosis between *PCR*+ and *PCR*-. *PCR*+ has a significantly lower spinal Z-score compared to *PCR*-. Further studies need to be performed prospectively with a

larger sample size adjusting for confounding factors such as presence of co-morbidities, use of steroids and anti-viral therapy.

P6

GENE THERAPY FOR MUSCULAR DYSTROPHY IN AN MDX MOUSE MODEL

S.D. McCarthy, K.J.A. McCullagh.
Department of Physiology, School of Medicine, and Regenerative Medicine Institute, National University of Ireland, Galway, Ireland.

Duchenne Muscular dystrophy (DMD) is the most common muscular dystrophy caused by loss of the sarcolemmal localised protein dystrophin. The deficiency in dystrophin causes the loss of the sarcolemmal membrane integrity and activates a cascade of cellular events that leads to degeneration of the muscle cell. Dystrophin is linked to several proteins including neuronal nitric oxide synthase (nNOS) which is involved in intracellular signalling. In DMD, nNOS expression is disrupted which is thought to contribute to the dystrophic pathology. Concomitantly, hypernitrosylation of the ryanodine receptor causes leakage of calcium from the endo/sarcoplasmic reticulum (ER/SR) in the *mdx* mouse[1]. Overexpression of sarcoplasmic Ca^{2+}-ATPase (SERCA) is reported to ameliorate the dystrophic phenotype in different models of muscular dystrophy[2]. Whether dystrophic muscle is subject to significant ER/SR stress has not been examined in any great detail. To this end, we investigated ER stress activation and the benefits of SERCA2a on dystrophic muscles in *mdx* mice.

We produced an adeno-associated-viral (AAV) muscle specific gene delivery system for delivering therapeutic molecules to the preclinical *mdx* mouse model of DMD. AAV vectors were prepared by triple transfection of 293T kidney cells with appropriate plasmids, followed by cell harvesting and virus purification. Initially, we generated an AAV2/9 viral vector with enhanced green fluorescent protein (eGFP), as the transgene, driven by the muscle specific spc512 promoter. We also generated a muscle specific nNOS vector and a CMV driven SERCA2a vector.

In vivo the AAV transgene vector was examined by both local delivery to the tibialis anterior (TA) muscle (IM) and via tail vein injection (IV). Animals were anaesthetised using inhaled isoflurane (0.08-1.5%). TA muscles were harvested for analysis by fluorescence, real-time RT-PCR and application of a GFP specific antibody. Highly efficient transduction of the TA muscle cells was observed 7 days post-exposure to AAV2/9-spc512-eGFP. Thus far we have developed a highly efficient *in vivo* muscle specific gene transduction system with successful AAV9spc5-12GFP (local and systemic delivery) and AAVcmvSERCA2a (local). However, delivery of nNOS and associated modulators is proving difficult.

We have observed an elevated ER stress response in the *mdx* model of DMD, which may be alleviated by over expression of the SERCA2a gene.

This work was supported by a grant (HRA/2009/79) from Health Research Board Ireland to KMC.

References

1. Bellinger A.M, Reiken S., Carlson C., Mongillo M., Liu X., Rothman L., Matecki S., Lacampagne A., Marks A.R. Hypernitrosylated ryanodine receptor calcium

release channels are leaky in dystrophic muscle. Nat. Med., 2009., Vol. 15. (3); 325-330.
2. Goonasekera S.A., Lam C.K., Millay D.P., Sargent M.A., Hajjar R.J., Kranias E.G., Molkentin J.D. Mitigation of muscular dystrophy in mice by SERCA overexpression in skeletal muscle. J. Clin. Invest. 2011., 121 (3), 1044–1052.

P7

CENTRAL INHIBITION OF FATTY ACID AMIDE HYDROLASE ATTENUATES TLR-3 INDUCED EXPRESSION OF INTERFERON-GAMMA AND RELATED GENES IN THE RAT HIPPOCAMPUS

R. Henry[1,3], D. Kerr[1,2,3], D.P. Finn[2,3], M. Roche[1,3]
[1]Physiology and [2]Pharmacology and Therapeutics, School of Medicine, [3]NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland, Galway, Ireland.

Interferon gamma (IFNγ) is considered to play a major role in the pathogenesis of several neurological disorders including Alzheimer's disease, Parkinson's disease and multiple sclerosis (1). Both exogenous and endogenous cannabinoids (endocannabinoids) have been shown to regulate IFNγ expression and/or signalling (2). However, the extent to which the brain's endocannabinoid system modulates the expression of IFNγ and related genes under conditions of acute neuroinflammation remains unknown.

The present study examined the effects of enhancing brain endocannabinoid tone using a selective inhibitor of fatty acid amide hydrolase (FAAH), the primary enzyme responsible for the metabolism of the endocannabinoid anandamide, on the expression of IFNγ and related genes in the rat hippocampus induced following toll-like receptor (TLR)-3 stimulation. Single guide cannulae were implanted into the lateral cerebral ventricles (i.c.v.) of adult male Sprague Dawley rats under isoflurane anaesthesia. Following a 6 day recovery period, animals (n=6-9 per group) received the FAAH inhibitor, URB597 (50µg, in 100% DMSO), or vehicle, i.c.v. at an infusion rate of 5µl/minute, 30 minutes prior to systemic administration of the TLR-3 agonist polyinosinic:polycytidylic acid (polyI:C:. 3mg/kg, i.p.) or sterile saline. Animals were sacrificed at 4 or 8 hours post polyI:C challenge, the hippocampus dissected out, snap-frozen and stored at -80°C. The expression of interleukin (IL)-12, IFNγ, iNOS IFNγ-inducible protein 10 (IP-10/CXCL10), and IL-10 were determined using qRT-PCR. Concentrations of the endocannabinoids, anandamide and 2-AG, and the related fatty acid ethanolamines, N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA), were determined using LC-MS-MS. Data were analysed using a 2-way ANOVA followed by Fisher's LSD *post-hoc* test. $P < 0.05$ was deemed significant.

Central administration of URB597 did not alter anandamide or 2-AG levels in the hippocampus, but significantly increased OEA (3.2 & 2.5 fold) and PEA (2.7 & 3 fold) concentrations at both 4 and 8 hours, respectively, post poly I:C administration. Systemic polyI:C administration increased the mRNA expression of IFNγ (7 fold), iNOS (8 fold), IP-10 (49 fold) and IL-10 (5 fold) at 4 hours and iNOS (6 fold), IP-10(11 fold) and IL-10 (7 fold) at 8 hours. Central administration of URB597 attenuated the polyI:C-induced expression of IFN-γ (2.25 fold) and IP-10 (1.4 fold) at 4 hours but did not alter the expression of IL-12, iNOS or IL-10 at either of the time points examined.

In conclusion, the present study demonstrates that enhancing levels of FAAH substrates, namely OEA and PEA, within the brain, attenuates the expression of IFNγ and related genes induced by TLR-3 activation. These data increase our understanding of FAAH-mediated regulation of IFNγ signalling in the brain and may aid in the identification of new therapeutic targets for various neuroinflammatory disorders.

Acknowledgements: Funding provided by Science Foundation Ireland Research Frontiers Project (Grant no. 11/RFP/NES/3175).

P8

THE NEURODEVELOPMENTAL AND BEHAVIOURAL EFFECTS OF METHAMPHETAMINE EXPOSURE DURING PREGNANCY IN RATS

K. McDonnell Dowling, V. MacEoin, S. Walsh, S. O'Brien, J.P. Kelly.
Discipline of Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, Ireland.

P9

DEVELOPMENT OF MINIATURIZED BIOMEDICAL SENSOR DEVICES

W. Messina[1], E. J. Moore[1,2]
[1]Sensing and Separation Group, Chemistry Department and Life Science Interface Group, Tyndall National Institute, University College Cork, Cork, and
[2]Department of Chemistry, University College Cork, Cork, Ireland.

Gold nanopillars have been successfully fabricated on top of interdigitated gold electrodes deposited on a Pyrex substrate and used for cytotoxicity monitoring through the electrochemical impedance spectroscopy technique on tissue cultures. These nanopillars have been fabricated via contact metal deposition. E-beam lithography has been used to define the pattern of nanopillars with dimensions of 150nm diameter and 500nm of distance between their edges in a honeycomb-like structure. These dimensions together with a low aspect ratio (50-60nm tall) have been chosen to possibly help promoting cell adhesion as these impedance electrodes will be used for tissue cultures testing of cytotoxicity. In this way we hope to increase the sensitivity of this kind of analysis compared to its plain counterpart.
Also, micro impedance sensor have been fabricated on the tip of a needle for determining the precise needle tip location prior to local anaesthetic injection, which remains a significant challenge during ultrasound-guided peripheral nerve block, despite widespread availability of high resolution ultrasound. Intraneural needle placement and local anaesthetic injection may be directly harmful to the peripheral nerve. Bioimpedance, as measured at the needle tip, may provide additional information as to needle tip location thereby potentially detecting intraneural needle position which could be in muscle, fat, connective tissue, blood vessels, etc, and with the help of the ultrasound techniques determine where the nerve is and not to damage it.

P10

RESOLVIN D1 (RVD1) INCREASES THE AIRWAY SURFACE LIQUID HEIGHT IN CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS

A. Moukachar[1,2], F. Ringholz[1], G. Higgins[1], V. Urbach[1].
[1]National Children's Research Centre, Crumlin, Dublin, Ireland, and [2]Université Pierre-et-Marie-Curie (UPMC), Paris VI, France.

Cystic Fibrosis (CF) is caused by mutation of the CFTR gene coding for a Cl^- channel and is characterized by ion transport abnormalities. In the airways the defective Cl^- secretion leads to reduced Airway Surface Liquid (ASL) height which favors chronic infection and sustained inflammation. The Docosahexaenoic acid derivative, Resolvin D1 (RvD1), is a specialized pro-resolution mediator binding either the FPR2 or GPR32 receptor (1) . ASL hydration by FPR2 agonist Lipoxin A_4 has previously been reported(2). We hypothesized that RvD1 increases the ASL height in normal and CF differentiated bronchial epithelial cells.

Non CF (NuLi-1) and CF (ΔF508/ΔF508, CuFi-1) cell lines were cultured as differentiated bronchial epithelial cells at air liquid interface (ALI). The ASL was labeled with Texas Red® Dextran and the ASL height was measured by live cell confocal fluorescence microscopy.

Under basal conditions, the non-CF Nuli-1 cells show an ASL height of 7.17±0.58 µM. As expected, in CF CuFi-1 cells the basal ASL height was significantly lower at 5.47±0.50 µM. Treatment of CuFi-1 cells using RvD1 restores the ASL height toward similar values measured in non-CF cells, without treatment. ResD1 for 30 minutes increased the ASL height by 1.50 µM (1nM) and 1.83 µM (100nM) in CuFi-1 cells ($P<0.05$ Student t-test).

In conclusion, these findings highlight novel therapeutic potential for RvD1 as a candidate adjunctive therapy for CF Lung disease by demonstrating to its ability to restore ASL hydration in addition to its recognized Pro-Resolution capabilities.

The authors acknowledge grant support from, the National Children Research centre and the French National Institute of Health (INSERM).

References

1. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. The Journal of experimental medicine. 2002;196(8):1025-37. Epub 2002/10/23.
2. Verrière V, Higgins G, Al-Alawi M, Costello RW, McNally P, Chiron R, et al. Lipoxin A_4 Stimulates Calcium-Activated Chloride Currents and Increases Airway Surface Liquid Height in Normal and Cystic Fibrosis Airway Epithelia. PloS one. 2012;7(5):e37746.

P11

CROSSTALK BETWEEN GLP-1 AND IL-6 IN ISOLATED RAT MYENTERIC AND SUBMUCOSAL NEURONS

A. Kelliher, L. O'Sullivan, M. Buckley, D. O'Malley.
Department of Physiology, University College Cork, Cork, Ireland.

Episodic bouts of bloating, abdominal pain and altered bowel habit are all characteristic of Irritable Bowel Syndrome (IBS). Underlying this debilitating disorder is brain-gut axis dysfunction but immune activation is also observed in IBS patients. Elevated circulating levels of the pro-inflammatory cytokine, interleukin (IL)-6 have resulted in it being proposed as a biomarker of IBS. In fact, IL-6 has functional consequences for GI activity, stimulating neuronal excitability and modulating colonic motility and secretion. Post-prandial exacerbation of IBS symptoms are commonly reported by patients but the underlying pathophysiology is not understood. A potential mediator of these symptoms is the incretin hormone glucagon-like peptide 1 (GLP-1), which is released from intestinal L-cells in response to nutrients.

This study aims to investigate potential areas of crosstalk between GLP-1 and IL-6 in IBS pathophysiology. Using whole mount *in vitro* preparations of colonic myenteric and submucosal neurons prepared from adult male Sprague Dawley rats, immunofluorescence dual-labelling techniques were used to investigate if GLP-1 receptors were expressed on IL-6 positive neurons. Calcium imaging was used to determine if GLP-1 has neuroexcitatory actions in each plexus. Finally, organ bath techniques were used to examine the functional effects of GLP-1 and IL-6 on colonic smooth muscle contractility.

Immunohistochemistry studies demonstrated the expression of GLP-1 receptors on fibres and ganglionic neurons in both plexi with ~30% of both submucosal and myenteric neurons co-expressing GLP-1 receptors and IL-6. In imaging studies, both GLP-1 and IL-6 independently evoked excitation of submucosal and myenteric neurons. Interestingly, motility studies found that although GLP-1 induced a robust contractile response in colonic strips, this was attenuated by co-application of IL-6.

These data demonstrate that about one third of colonic enteric neurons express both GLP-1 receptors and IL-6. Moreover, both GLP-1 and IL-6 can stimulate a subset of enteric neurons causing an increase in intracellular calcium. Consistent with the role of myenteric neurons in regulating gastrointestinal motility, GLP-1 evokes robust contractile activity in colonic sections. However, this effect is inhibited by the presence of IL-6. These data provide evidence for crosstalk between GLP-1 and IL-6 in colonic function and this may be important in the post-prandial exacerbation of IBS symptoms when both IL-6 and GLP-1 levels are elevated.

P12

THE EFFECT OF FOAM ROLLING ON SUBSEQUENT EXERCISE PERFORMANCE IN MAN

J. Bradley[1], J. Gomez[2], T. Woods[3].
[1]School of Education, [2]Mardyke Arena and [3]Department of Sport & Physical Activity, University College Cork, Cork, Ireland.

Foam Rolling (FR), also called self-myofascial release (SMR) and Static Stretching (SS) are increasingly practised by athletes to treat soft-tissue restrictions

and increase Range of Motion (RoM). Recent research has suggested reduced muscle performance from SS[1]. The objectives of this study were to compare FR, SS and a similar duration of Dynamic Mobility (DM) on subsequent explosive muscle performance.

Nine subjects (5 males, 4 females; Age: 28.0±6.8years; Weight: 76.9±8.5kg) volunteered for the study. Following an initial familiarisation visit, subjects carried out muscle performance exercises prior to and following one of three different conditions: FR (2x30sec on each of quadriceps, hamstrings and plantar flexor muscle groups with 30sec rest); SS (2x30sec to the point of discomfort on each of quadriceps, hamstrings and plantar flexor muscle groups with 30sec rest); DM (6 minutes of cycle exercise on a cycle ergometer at 70W). The exercise condition was randomised. Muscle performance was assessed using Vertical Jump (VJ) height and a Counter Movement Drop Jump height and ground contact time (CMJ, from height of 24cm). All four visits were at the same time of day, on the same day of the week over three successive weeks. The results were analysed using ANOVA with LSD post hoc tests. Significance was assumed at $P<0.05$.

SS caused a greater reduction in VJ height and CMJ height compared to DM and FR (Post-Pre condition change: 4.4±2.2cm vs. 2.2±1.8cm* vs. 2.3±0.8cm* and 5.6±4.1cm vs. 4.5±1.7cm vs. 1.9±3.4cm* respectively; *$P<0.05$). FR resulted in a greater CMJ contact time compared to SS and DM (7.0±24.3ms vs. -36.8±25.1ms* vs. -33.5±18.9ms* respectively). This is consistent with studies showing reduced jumping performance from a variety of static stretching styles and intensities[1]. FR resulted in no significant change in CMJ height and contact time.

High intensity SS increases RoM through reducing musculotendinous unit (MTU) stiffness but has a detrimental impact on force transmission due to reduced neuromuscular activation. This will result in reduced jump height or increased ground contact time. The unchanged CMJ height and contact time from FR suggest SMR has a different effect to static stretching. Recent evidence has suggested the increased RoM from short duration SMR is due to enhanced thixotropic properties of the fascia enveloping the muscle rather than placing pressure on the origin and insertion points of the muscle[2]. Short periods of foam rolling may therefore enhance the soft tissue pliability, increasing RoM without impacting on neuromuscular activation or explosive muscle performance.

References

1. Behm, DG & Kibele, A (2007). Effects of differing intensities of static stretching on jump performance. Eur J Appl Physio 101, 587-594.
2. MacDonald, GZ, Penney, MD, Mullaley, ME, Cuconato, AL, Drake, CD, Behm, DG & Button, DC (2013). An acute bout of self myofascial release increases range of motion without a subsequent decrease in muscle activation or force. J Strength Cond Res 27(3), 812-821.

P13

SMALL-MOLECULE INHIBITORS AT THE PSD-95/nNOS INTERFACE HAVE ANTIDEPRESSANT-LIKE PROPERTIES IN MICE

M. V. Doucet[1,2], H. Levine[1], K. K. Dev[2], A. Harkin[1].

Previous studies have demonstrated that nitric oxide (NO) synthase inhibitors are as efficacious as tricyclic antidepressants in preclinical antidepressant screening procedures and in attenuating behavioural deficits associated with animal models of depression. The N-methyl-D-aspartate receptor (NMDA-R) complex gates Ca^{2+}, which binds to the calmodulin domain of the nitric oxide (NO) synthase enzyme to subsequently activate NO synthase. We hypothesised that uncoupling nNOS from the NMDA-R through the scaffolding protein PSD-95 would produce behavioural antidepressant effects similar to NO synthase inhibitors.

Small-molecule inhibitors of the PSD-95/nNOS interaction, IC87201 (0.01-2 mg/kg) and ZL006 (10 mg/kg) were tested for antidepressant properties in tests of antidepressant activity, namely the tail suspension and forced swim tests in mice. We now report that IC87201 and ZL006 produce antidepressant-like responses in the FST and TST following a single administration in mice. By contrast to the tricyclic antidepressant imipramine (25 mg/kg) the effects are not observed 1h following drug administration but were apparent 24h and 72h later. Furthermore prior exposure to the TST or FST is required in order to observe the antidepressant-related activity. Similar delayed and sustained antidepressant-like effects were observed following TRIM (50 mg/kg) and ketamine (30 mg/kg) in the TST. The antidepressant-like effects of ZL006 also generalise to IC87201 in the TST. IC87201 was devoid of effects on locomotor activity and step-through latency in the passive avoidance cognition test.

These data support the hypothesis that targeting the PSD-95/nNOS interaction downstream of NMDA-R produces antidepressant effects and may represent a novel class of therapeutics for the treatment of major depression.

The authors acknowledge grant support from the Health Research Board of Ireland.

P14

BREAST CANCER AND DUCTAL CARCINOMA *IN SITU* IDENTIFICATION USING A DUAL-ELECTRODE SYSTEM

N.T.P. Savage[1], B.D. O'Donnell[2], M.J. O'Sullivan[2], E.J. Moore[1]

[1]Sensing and Separation Group, Department of Chemistry and Life Science Interface Group, Tyndall National Institute, University College Cork and [2]BreastCheck and Cork University Hospital, Cork, Ireland.

Breast cancer is the term used to categorise a diverse range of oncologic conditions which can affect both the ducts and lobules of the breast. Prior to the invasion of the surrounding healthy tissue many breast cancers develop initially as ductal carcinoma *in situ* (DCIS) which is a treatable pre-cancerous condition that increases the lifetime risk of breast cancer by as much as 20% [1]. The aim of this project is to create a novel electrical impedance device for the detection of both invasive breast carcinomas (IBC) and precancerous DCIS. Improved identification of DCIS lesions could allow earlier treatment (which results in a 95 % survival rate) and therefore prevent the invasion of these cells beyond the basement membrane and

the possible metastatic spread of the disease to the bones, liver and lungs which are the most common sites associated with secondary breast cancer.

Electrical impedance measures the opposition that a circuit presents to a current when a voltage is applied. Each cell type in the human body is composed of different chemical and physical elements which result in characteristic impedance signals generated by each cell variant [2]. Our plan is to use the process of photolithography to pattern dual micro-structured electrodes onto the tip of a hypodermic needle. By applying a minute current across these electrodes it will allow real-time identification of cell populations as either healthy or cancerous. This technique will be used to determine the electrical impedance of both cell culture models (BT-20 and MCF-7a immortalised cell lines) and excisional human biopsy samples (that have been pathologically defined). All of the fabrication processes and materials used to create the device will result in it being biocompatible with human tissues. This will mean it could be inserted directly into the regions of interest within a patient's breast as identified by areas of micro-calcifications (DCIS) or radiographic-density (IBC) on a diagnostic mammogram. There are a number of potential uses for this device including improved biopsy localisation, cancer-free border determination during lumpectomy and the possibility of DCIS determination without invasive surgery.

The authors acknowledge grant support from Molecular Medicine Ireland as part of the Clinical & Translational Research Scholars Programme.

References

1. American Cancer Society (2012) Breast Cancer Facts & Figures 2011-2012. Atlanta: American Cancer Society, Inc.
2. Morimoto T, Kimura S, Konishi Y, Komaki K, Uyama T, Monden Y, Kinouchi Y, Iritani T (1993) A study of the electrical bio-impedance of tumors. J Invest Surg 6:25-32.

P15

CELL BASED BIOSENSORS FOR USE IN CYTOTOXICITY STUDIES

M. Fitzgerald[1], E.J. Moore[1]
[1]Sensing and Separation Group, Department of Chemistry and Life Science Interface Group, Tyndall National Institute, University College Cork, Ireland.

Cell based biosensors are measurement devices which use mammalian cells as the sensing element. The response of the cells provides an understanding of the effects of the analyte at a physiological level[1]. It has long been acknowledged that the phospholipid bilayer of the plasma membrane possesses dielectric properties. Culturing cells over electrode contacts and measuring changes in the effective electrode impedance offers a non-invasive assay to determine the adhesion, spreading, and motility of a cultured cell line. Impedance measurements are centred on the fact that whole live cells, at low signal frequencies, are excellent electrical insulators. Cell growth and migration leading to increased coverage of an electrode surface result in increased electrode impedance[1]. Upon exposure to a substance which adversely affects their health, adherent cells will detach from the electrode surface[2]. A change in impedance value is therefore seen to be directly related to

attachment and spreading of cells on the surface of the electrodes. These changes in impedance values can be used to explain cell behaviour and to test new drug substances[2]. Over the last decade, there has been growing interest in the use of biosensors in a large range of applications including; environmental, medical, toxicological, and defence[1].

Toxichip is an example of a successful project based on these theories. It functions as an integrated eukaryotic cell-based micro-sensor platform that examines the effects of toxic chemicals on cells. The system allows for multi-parameter sensing including pH, temperature, and dissolved oxygen levels to ensure cells are maintained at optimum conditions. Cells are cultured on the inter-digitated electrode structures. Upon attachment and spreading of cells, the impedance increases because the cells act as insulating particles to restrict the current flow. Integration of biosensors with a fluidic platform enables real time simultaneous monitoring of cellular behaviour using both optical and electrical detection. This type of system has applications in various sectors such as: health, pharmaceutical, cosmetic, environmental, security, & food/beverage.

References

1. Pancrazio, J.J., Whelan J. P., Borkholder D.A., Ma W., Stenger D.A., "Development and application of cell-based biosensors." Annals of Biomedical Engineering 27, no. 6 (1999): 697-711.
2. Mijares G.I., Reyes D.R., Geist J., Gaitan M, Polk B.J., DeVoe D.L., "Polyelectrolyte Multilayer-Treated Electrodes for Real-Time Electronic Sensing of Cell Proliferation." Journal of Research of the National Institute of Standards and Technology 115, no. 2 (2010).

P16

EVIDENCE FOR THE INVOLVEMENT OF CYCLIC ADP RIBOSE-DEPENDENT ENDOPLASMIC RETICULUM Ca^{2+} RELEASE BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS.

J. Leahy, A. Kaar, M.G. Rae

Department of Physiology, University College Cork, Cork, Ireland.

Group 1 metabotropic glutamate receptors (I-mGluRs) are important neuromodulators of synaptic plasticity and also play a role in aetiology of Alzheimer's disease. For many years I-mGluRs have been thought to mediate their effects primarily by evoking the mobilisation of intracellular calcium from the neuronal endoplasmic reticulum (ER) *via* the production of inositol-1,4,5 - trisphosphate (IP_3), catalysed by the activation of phospholipase Cβ□ (PLC). However, recent work suggests that in acutely dissociated rat hippocampal neurons I-mGluR-mediated intracellular Ca^{2+} mobilisation is at least partly dependent on cyclic ADP ribose (cADPR) - dependent activation of ryanodine receptors (RyRs)[1]. It was the aim, therefore, of the current study to determine if I-mGluR –mediated Ca^{2+} signals within cultured rat hippocampal neurons, previously ascribed to activation of the PLC/IP_3 signalling pathway [2, 3], may be mediated *via* the generation of cADPR.

Experiments were carried out utilising cultured hippocampal neurones obtained from 3-4 day old Sprague Dawley rat pups as previously described [2]. Conventional calcium imaging was used to record intracellular somatic calcium

measurements from cells loaded with the calcium sensitive dye, fluo -2 AM (150 μM). Experiments were carried out at room temperature with neurons continuously perfused (2ml/min) with a standard HEPES-buffered saline solution (HBSS). All drugs were added to the perfusate. Data are expressed as means ± S.E.M.

Stimulation of I-mGluRs, using the specific agonist, (*S*)-3, 5 - dihydroxyphenylglycine (DHPG; 50 μM; 2 min) under control conditions evoked somatic $[Ca^{2+}]_i$ signals of 4678 ± 503 units (n = 60) (as determined by measuring area under the curve). Following depolarisation with elevated extracellular K^+ (15 mM) - containing HBSS solution, DHPG- evoked $[Ca^{2+}]_i$ signals were significantly enhanced relative to controls by 26.1 ± 9.5% (P<0.05; n=60) in line with previous studies on rat hippocampal neurones. Application of the cADPR antagonist nicotinamide (5 mM) significantly reduced the amplitude of DHPG- evoked $[Ca^{2+}]_i$ signals elicited in 15 mM K^+-HBSS by 23.8 ± 14.7 (n= 34; P < 0.01).

Application of the IP_3R antagonist, 2-APB (250 μM), did not significantly affect DHPG- evoked $[Ca^{2+}]_i$ (n = 17; P = 0.97). However, the PLC inhibitor, U73122 (5 μM), did significantly inhibit the DHPG- evoked $[Ca^{2+}]_i$ signals by 56.5 ± 16.3% (n = 9; P < 0.05).

The nicotinamide and 2-APB experiments support the suggestion that I-mGluR-evoked $[Ca^{2+}]_i$ signals may be mediated, at least in part, *via* cADPR-dependent signalling. However, the majority of the DHPG-evoked response is still mediated primarily by generation of IP_3 as evidenced by the effect of the PLC inhibitor U73122.

References

1. Sohn *et al.,* (2011) Cyclic ADP ribose-dependent Ca2+ release by group I metabotropic glutamate receptors in acutely dissociated rat hippocampal neurons. *Plos One*, 6 (10), e26625.
2. Rae, M.G. & Irving, A.J. (2000) *J. Neurosci.,* 20 (23), 8628-8636.

P17

ESTABLISHMENT OF THE GUT MICROBIOME DURING EARLY LIFE INFLUENCES THE SURVIVAL OF NEWLY-BORN CELLS IN THE ADULT HIPPOCAMPUS

S. Ogbonnaya[1,3], G. Clarke[2,4], F. Shanahan[2], J.F. Cryan[1,2], O.F. O'Leary[1].

[1]Department of Anatomy and Neuroscience, [2]Alimentary Pharmabiotic Centre, University College Cork, [3]Department of Neurosurgery, Cork University Hospital, Ireland, and [4]Department of Psychiatry, University College Cork, Ireland

Accumulating evidence suggests that the gut microbiome can influence behaviour and brain function. In particular, germ-free mice exhibit reduced anxiety and this phenotype is accompanied by alterations in hippocampal concentrations of serotonin and brain-derived neurotrophic factor (BDNF), substrates that are important in the regulation of anxiety. BDNF and serotonin are also key regulators of adult hippocampal neurogenesis, a process that has been implicated in the regulation of anxiety and various cognitive processes including spatial learning and memory. Exactly how adult hippocampal neurogenesis plays a role in diverse functions such as anxiety and spatial learning and memory, is currently unknown, but accumulating evidence suggests that the hippocampus is functionally segregated into dorsal (dHi) and ventral (vHi) regions and that this might also apply

to neurogenesis. Specifically, the dHi appears to play a preferential role in spatial learning & memory while the vHi plays a preferential role in anxiety. Given that several substrates that regulate adult hippocampal neurogenesis are altered in germ-free mice, it is plausible that adult hippocampal neurogenesis might also be affected in these mice. Therefore, the aim of the present study was to determine whether the proliferation and survival of newly-born cells along the dorso-ventral axis of the adult hippocampus is altered in germ-free (GF) mice compared to conventionally colonised (CC) mice. Data is expressed as mean ± S.E.M., and was analysed using one way analysis of variance (ANOVA).

Compared with CC mice, GF mice exhibited increased survival of newly-born cells in the dHi ($p = 0.008$), but not vHi, and these effects were not reversed by bacterial colonization of GF mice post weaning. This suggests that the gut microbiome can influence the survival of newly-born cells in the adult hippocampus, and that there is a critical period during development when this occurs. Whether these cells differentiate into neurons is currently being investigated. Moreover, the specific effect in the dHi raises the possibility that GF might exhibit altered spatial memory capabilities.

P18

THE IMPACT OF HIGH SALT DIET ON THE REGULATION OF RENAL HEMODYNAMICS AND NITRIC OXIDE IN WISTAR RATS

H. F. Shabana, A. F. Ahmeda, E. J. Johns.
Department of Physiology, University College Cork, Ireland.

Nitric oxide (NO) is involved in controlling the medullary blood perfusion and arterial blood pressure (BP). NO interacts with locally produced superoxide anions to produce peroxynitrite, a cytotoxic molecule. A high sodium (HNa^+) diet is associated with decreased levels of NO metabolites and increased superoxide anions. These factors might modulate the tone of the renal microvasculature and cause hypertension. This study investigated whether NO regulates the cortical and medullary perfusion (CP and MP, respectively) in HNa^+ rats.

This was done by blocking NOS activity in both normal sodium (NNa^+) or HNa^+ fed rats. Groups (n=7-8) of male Wistar rats were fed NNa^+ (0.4%) or HNa^+ (4%) diet for two weeks. Rats were anaesthetized with 1ml i.p Chloralose/Urethane.
The right femoral vein was cannulated for infusion of saline (154mM NaCl) at 3ml/h and supplemental doses of anaesthetic. The right femoral artery was cannulated for measurement of BP and heart rate (HR). Through a flank incision, the left kidney was exposed and supported in a cup holder to eliminate respiratory movement and a small cannula was inserted 4.5mm into the kidney for intramedullary (i-m) infusion of saline or drugs at 0.6-1.0 ml/h. Two Laser-Doppler microprobes (each 0.5 mm diameter) were inserted 1.5 and 4.0 mm into the kidney to measure cortical and medullary perfusion, respectively. After 90min, baseline measurements were taken, and then L-NAME (Nitric oxide synthase, NOS, inhibitor) was infused i-m for 30 min at 10 µg/kg/min. Data, means ± SEM, were subjected to the Student's t-test and significance taken at $P<0.05$.

The HNa+ group had a significantly ($p<0.05$) lower baseline level of MP, 47 ± 2 PU than the NNa+ which was 67 ± 1.8 PU. The CP shows no significant reduction in HNa+ compared with NNa+ (183± 5PU V 223± 9PU). Neither BP nor HR was affected in either group. Infusion of L-NAME into the normal group caused

a reduction in MP 88±2% (P< 0.05), whereas a decrease in both CP & MP reaching 84±4% and 89±2%, respectively (p< 0.05) in HNa+ group. The BP of the two groups rose significantly (P< 0.05) after L-NAME infusion to reach 105±1.5%mmHg and 112±3%mmHg, respectively. The HR of both groups was reduced from the baseline values after L-NAME infusion, however, this was only significant in the HNa^+ group.

The findings demonstrate that blocking the production of NO in the presence of oxidative stress as a result of HNa^+ intake was associated with a reduction in medullary and cortical perfusion. NO plays an important role as a vasodilator in regulating the tone of medullary blood vessel to maintain normal BP. HNa+ diet may diminish the NO production which is reflected by the changes in BP and HR after L-NAME infusion.

P19

PROLIFERATION OF HEPATIC STELLATE CELLS FOLLOWING TRANSPLANTATION OF THE LIVER: AN INTRAVITAL FLUORESCENCE MICROSCOPY STUDY

R. C. Chigozie[1], C. Prendergast[1], M. Tawadrous[2], A.M. Wheatley[1,2].
[1]Department of Physiology, School of Medicine, NUI Galway, Ireland
and [2]Department of Physiology, University of Otago, New Zealand.

Hepatic stellate cells (HSCs) are known to play a role in fibrotic liver disorders (1), however their response to injury sustained due to liver transplantation is not well established. Activation of HSCs following liver transplantation may occur as a result of acute rejection of hepatic allografts. The objective of this study was to investigate the proliferation of HSCs following orthotopic liver transplantation (OLT) in the rat using intravital fluorescent microscopy (IVFM) and the impact immunosuppression has on any HSC proliferation.

Rat OLT with graft rearterialisation was carried out under ether anaesthesia in Lewis-Lewis (donor-recipient) or DA-Lewis rats. We investigated (a) the effect of acute rejection and CsA treatment (10mg/kg/day) on HSC proliferation and (b) the effect of FTY720 alone (1mg/kg/day) or in combination with CsA (5mg/kg/day) on HSC proliferation. At days 2, 4 and 7 after OLT under barbiturate anaesthesia, the liver was subjected to IVFM. Visualisation of vitamin A autofluorescence associated with HSCs was achieved using a 330-390/450nm filter combination (excitation/emission) and were quantified off-line using the Image J software.

Our results showed that (1) untreated acute rejection is associated with increasing HSC proliferation (cell number and area) from day 2 to 7 following liver transplantation (2) this HSC proliferation is attenuated by CsA-treatment ($p < 0.01$) (3) Treatment with FTY720 alone or in combination with CsA causes a reduction in HSC proliferation and there was no significant difference in HSC proliferation between both treatment regimens ($p > 0.05$) .

HSC proliferation occurs during acute rejection of the transplanted liver which is greatly reduced by immunosuppressive therapy. Further investigations is required to elucidate the underlying mechanisms by which HSCs become proliferative in response to transplantation-induced injury.

References

1. Moreira RK, Hepatic stellate cells and liver fibrosis, Arch Pathol Lab Med: 131, 1728-1734 (2007).
2. Tawadrous MN, Persistence of impaired hepatic microcirculation after nonarterialized liver transplantation in the rat. Microcirculation 9, 363-75.

P20

A PROSPECTIVE AUDIT OF HAEMATINIC REQUESTS FROM GENERAL PRACTICE IN CORK UNIVERSITY HOSPITAL (CUH) HAEMATOLOGY LABORATORY

M. O'Meara[1], S. Cadogan[2], M. O'Reilly[2], D. Minihane[2], M.R. Cahill[2]
[1]Department of Biochemistry, St James' Hospital, Dublin 8 and [2]Department of Haematology, Cork University Hospital, Cork, Ireland.

In 2012 Cork University Hospital (CUH) haematology laboratory processed approximately 8,000 test requests for haematinics each week, with 409,343 test requests (>145,000 samples) processed by year end. 31% of these were from Kerry General Hospital (KGH). The haematology laboratory provides haematinic testing to a population of 664,534 (based on the Irish census 2011). A previous audit found that 80% of clinical details did not contain clinical details, with 5% of requests on normal results within 3 months. Our objective was to develop, through audit, a strategy to optimise haematinic testing in HSE South in an area where test numbers are rising yearly with no defined increase in clinical need. Aims of the audit included identifying for each request (1) The nature of the clinical indication and (2) The degree of abnormality associated with that indication. Laboratories have an important educational role and we aimed to give feedback on the findings from this audit to our clinical users.

In February 2012 we carried out a prospective audit of 1077 request forms selected at random from General Practice (GP) over a 7 day period. Those laboratory request forms without clinical details were put to one side and the respective GP practices contacted for details. We used background data on population prevalence for haematinic to make comparisons on the clinical utility of requesting.

276 had no details (26%). Deficiency rates of 3.3%, 3.7% and 11% for B12, folate and ferritin respectively age range 1–100 years (mean age 51). We note 12% repeats within 3 months, with 7% repeat request rate on normal results; 18 patients >3 samples sent from the same practice. Of the 76 requests with haemochromatosis as indication; 45/76 also requested for B12 and 38/76 for folate. Under 24 year olds displayed the highest iron deficiency rate–24% in this category were deficient. Of the 1043 ferritin requests received; 5% (55) were >320 pmol/l, and 0.006% (6) were >1000 pmol/l. The most common indications were 'screening', 'fatigue' and 'on replacement'; eighty-seven percent of these requests showed normal results.

British Committee for Standards in Haematology guidelines on B12 and folate requesting are due for publication mid- 2013. Or audit has shown that although the tests are not expensive, more judicious requesting by all requesters of haematinics laboratory savings could be effected. New requesting guidelines suggesting significant requesting changes may be safely recommended to GPs.

P21

BILATERAL MICROINJECTION OF THE CB1 RECEPTOR AGONIST ACEA INTO THE PAG DIFFERENTIALLY MODULATES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR IN SPRAGUE-DAWLEY AND STRESS HYPER-RESPONSIVE WISTAR-KYOTO RATS

E. M. Jennings [1,2] , K. Rea [1,2] B. Okine [1,2], F. McGowan[1], M. Roche [2,3], D.P. Finn [1,2]

[1] Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, [2] NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland, Galway, and [3]Physiology, School of Medicine, National University of Ireland, Galway, Ireland.

P22

INHIBITORY ACTION OF THE TRPA1 ANTAGONIST, HC-030031, ON RAT TRPM8

C. Doran, G. Reid.

Department of Physiology, University College Cork, Ireland.

HC-030031 is an antagonist for the irritant ion channel receptor TRPA1 (1). Given the controversial role of both TRPA1 and the cool receptor, TRPM8, in cold induced nociception, and increasing evidence of their shared pharmacology, the compound has been used in a number of studies to discriminate between these two channels. Here we show that HC-030031 also antagonises rat TRPM8, either heterologously expressed in HEK293 cells or natively expressed in rat dorsal root ganglia (DRG), and is not specific for TRPA1 as has been assumed in other studies.

Male Sprague-Dawley rats (100-150 g) were killed by CO_2 inhalation and decapitation. DRG neurones were cultured for 12-36 hours in the presence of NGF. Cloned rat TRPM8 was transiently transfected in HEK293 cells using Fugene 6. Responses to compounds were evaluated by calcium microfluorimetry using Calcium Green 1-AM ($\Delta F/F_0$). Data are presented as mean ± SEM and analysed by the Student's paired t test (one–tailed) unless otherwise stated.

Menthol activates TRPM8 and has a bimodal effect on mouse TRPA1, activating the channel over a narrow concentration range (2). HC-030031 (20 μM) inhibited the responses of menthol-sensitive rat DRG neurones over a range of concentrations ($\Delta F/F_0$ = 0.13± 0.03 versus control 0.33 ± 0.4, $p< 0.0001$ n=294) including those sufficiently high to activate TRPM8 alone (200 and 300 μM). Significantly fewer DRG neurones were activated by 300 μM menthol, consistent with TRPA1 inhibition at this concentration (19.1 % versus 34 %, 300 and 100 μM respectively, p=0.0093, Fisher's Exact Test). The TRPM8-selective agonist, WS-12 (40 μM) activated a similar percentage of neurones to 300 μM menthol (22%). HC-030031 completely inhibited responses to WS-12 in 50 % of neurones and blocked responses overall (0.096 ± 0.03, versus 0.193 ± 0.03; $p<0.0001$, n=122). Recombinant rat TRPM8 responses to cold were attenuated in the presence of HC-030031 (0.1426 ± 0.017 versus 0.2769 ± 0.026, $p<0.0001$, n=37).

HC-030031 is used as a specific blocker of TRPA1 and has been proposed as a therapeutic candidate in numerous pathological conditions. However, the evidence reported here suggests that the consequences of its off target actions on TRPM8 need to be better understood.

References

1. McNamara C et al., Proc Natl Acad Sci USA. 2007;104:13525 - 30.
2. Karashima Y et al., J Neurosci 2007; 27:9874-84.

P23

CAMPHOR ACTIVATES AND SENSITIZES RAT AND HUMAN TRPM8 TO ICILIN

T. Selescu[1,2], C. Ciobanu[1,2], C. Dobre[1], G. Reid[2], A. Babes[1].
[1]Department of Anatomy, Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania and [2]Department of Physiology, University College Cork, Ireland.

The ion channel TRPM8 is activated by cooling and chemical stimuli including menthol, eucalyptol and icilin.

Human TRPM8 (hTRPM8) was stably expressed in HEK293 cells (hTRPM8-HEK293). Adult male Wistar rats were decapitated after CO_2 inhalation, and dorsal root ganglion (DRG) neuron cultures prepared. Intracellular calcium was imaged with Calcium Green-1 at 25°C. Whole-cell currents were recorded with conventional or perforated patch recording. Ruthenium Red (10 µM, extracellular), a blocker of the other ion channels activated by camphor (TRPV1, TRPV3) or icilin (TRPA1), was present in all experiments on DRG neurons. Data are presented as mean ± SD and significance tested with Student's paired t-test.

In DRG neurons camphor activates cold- and icilin-sensitive neurons (characteristics of TRPM8 expression) [1]. In calcium imaging experiments, camphor activated recombinant hTRPM8 (EC_{50}=4.48 mM, n=59) in a temperature-dependent manner. Currents elicited by 10 mM camphor in perforated patch clamp experiments (47.3±27.2 pA/pF, n=8) on hTRPM8-HEK293 were larger and displayed much less tachyphylaxis than those recorded in conventional whole-cell mode (18.8±30.5 pA/pF, n=12), suggesting modulation by a cytosolic factor.

Camphor markedly enhanced the response to icilin in both hTRPM8-HEK293 and cold-sensitive rat DRG neurons. The whole-cell currents displayed supra-additive behaviour and the effect was calcium-dependent. In hTRPM8-HEK293, camphor (10 mM) enhanced the icilin (2 µM)-elicited currents from 25.0±20.8 to 119.2±19.8 pA/pF (n=4, $p<0.01$). Camphor (5 mM) strongly and reversibly inhibited the whole-cell currents elicited by menthol (100 µM) in hTRPM8-HEK293, by 68±20% at -60 mV (n=7, $p<0.001$) and by 85±17% at +80mV (n=7, $p<0.001$).

The icilin-insensitive chick orthologue of TRPM8 (cTRPM8) was camphor- and eucalyptol-insensitive, but these two compounds still inhibited cTRPM8's response to menthol. In contrast, the icilin-insensitive hTRPM8 mutant D802A retained the camphor sensitivity of the wild-type, indicating that icilin and camphor sensitivity of hTRPM8 depend on distinct sites within the molecule.

Supported by the European Social Fund [POSDRU 107/1.5/S/80765 to T.S.]; the Romanian Research Council (CNCS) [PCE 117/2011 to C.D., A.C.C. and A.B.]; and Science Foundation Ireland [BIM085 to G.R.].

References

1. Selescu T, Ciobanu C, Dobre C, Reid G, Babes A. Chemical Senses, in press.

P24

CHICK, BUT NOT RAT, SENSORY NEURONES EXPRESS THE WARM-ACTIVATED ION CHANNEL TRPV3

C. Doran, N. Morshed, G. Reid
Department of Physiology, University College Cork, Ireland.

Innocuous cooling and noxious heating of the skin are detected by sensory neurones expressing "thermo-TRP" ion channels (1). Innocuous warming may also be detected by a member of this family, TRPV3. This channel is expressed in human sensory neurones, but is absent from those of mice, which have been the model for virtually all the research up to now on TRPV3; in mice, it is expressed in skin keratinocytes.

We have previously shown that dorsal root ganglion (DRG) neurones from domestic chicks respond to warming with TRPV3-like thermal thresholds and pharmacology (2). In that study, we showed that 7.6 % of neurones responded to 38 °C warming ramps with increases in intracellular Ca^{2+}, and these neurones were activated by camphor (an agonist of cTRPV3 but not cTRPV1).

To clarify the molecular basis for this, we have investigated the expression of TRPV3 in chick and rat DRG using qPCR.

Hatchling chicks were killed by decapitation, and male Sprague-Dawley rats (150-200 g) were killed by CO_2 inhalation followed by decapitation. DRG neurones from all spinal levels were removed for immediate total RNA extraction (Trizol) and cDNA synthesis (Promega). Bryt green®-based qPCR was performed on an Illumina Eco thermocycler. Expression of cTRPV3 and rTRPV3 was measured against two reference genes: the neurone specific TUBB3, and β-actin.

We found TRPV3 to be expressed in chick DRG, at lower levels than the reference genes (PCR efficiencies of 102.55, 98.08 and 90.77 % for TRPV3, TUBB3 and β-actin respectively), consistent with functional responses and expression in ~7 % of chick DRG neurones. In contrast, in rat DRG, TRPV3 was undetectable, although both reference genes were successfully amplified at levels similar to those in chick.

We conclude that chicks contain substantial numbers of warm-sensitive DRG neurones and that TRPV3, expressed in chick DRG, is able to explain their thermosensitivity. In contrast, as reported in mice, TRPV3 is absent from rat DRG. Given that TRPV3 is expressed in human DRG, we therefore suggest that chicks may be a more appropriate model than rodents to understand peripheral warm sensing in humans and the function of TRPV3 in sensory neurones.

Funding was from the Department of Physiology, UCC.

References

1. Patapoutian, A. et al. (2003) Nature Rev. Neurosci. 4:529-539
2. Reid, G. (2005) J. Physiol. 567P:C42.

P25

TRPV1 BLOCKADE RESTORES THE CARDIOPULMONARY BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY IN CISPLATIN-INDUCED RENAL FAILURE RATS

M. Duff[1], M.H. Abdulla[1], E. J. Johns[1,2]. [1]Department of Physiology, University College Cork, Ireland and [2]Allianze University, College of Medical Sciences, Penang, Malaysia.

The transient receptor potential vanilloid type 1 (TRPV1) is a key signalling channel for sensory nerves including those originating in the kidney. The current study investigated whether TRPV1 contributes to the modulation of low-pressure baroreceptor reflex regulation of renal sympathetic nerve activity (RSNA) in a rat model of cisplatin-induced renal failure.

Wistar rats (290-350g) were divided into a renal failure (RF, n=7) group, which received IP cisplatin (5mg/kg), and a control (C, n=7) group which received IP saline (5ml/kg, 0.9% NaCl) four days before the acute study. Rats were anaesthetised with chloralose:urethane mixture (1-1.5ml, 16.5:250mg/ml) IP. Cannulae were inserted into the right femoral artery, for mean arterial pressure (MAP) and heart rate (HR) measurement, and vein for saline (3ml/h) infusion. The right kidney was exposed and a small cannula inserted 4.5mm into the rostral pole of the kidney for intrarenal infusion of saline or capsazepine (CPZ). The left kidney was exposed, a renal sympathetic nerve dissected and sealed onto recording electrodes. Following a 2 h stabilisation, the rats were subjected to volume expansion (VEP, 0.25% bwt/min saline for 30min IV). Saline or CPZ (5µg/ml) was infused intracorticomedullary to the right kidney 20min before, and during the first and second VEP respectively at 1ml/h. The animal was euthanized and the background noise was obtained and subtracted from all the readings. Data, mean±SEM were compared using *t*-test with significance at $P<0.05$.

CPZ infusion in C or RF groups, did not change baseline MAP, HR or RSNA (*CPZ vs. baseline*; C, 89±2 vs. 84±2mmHg, 347±16 vs. 344±10bpm, 1.47±0.33 vs. 1.36±0.41µV.s; RF, 83±5 vs. 80±3mmHg, 310±10 vs. 337±14bpm, 1.60±0.38 vs. 1.33±0.34µV.s, respectively). The VEP caused a significant reduction of 33±2% ($P<0.05$) in RSNA in C whereas in RF the renal sympatho-inhibition was significantly ($P<0.05$) less 10±4%. In C, the CPZ had no effect on the magnitude of the VEP induced reduction in RSNA, at 38±7%, but in RF, the magnitude of the renal sympatho-inhibition was enhanced (33±5 vs. 10±4%, $P<0.05$). These findings demonstrate that under normal conditions, TRPV1 channels contribute little to the low- pressure reflex regulation of RSNA. By contrast, in cisplatin-induced renal failure there is an inappropriate activation of the TRPV1 which prevents the reflex renal sympatho-inhibition to VEP.

These findings highlight the significant control that renal afferent nerves may exert in determining sympathetic outflow in renal disease and injury.

This work was funded by the Wellcome Trust.

P26

THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA CO-ACTIVATOR 1-ALPHA IN PREGNANCY

A.C. Delany[1], S.K. Walsh[2], L.C. Kenny[3], F.P. McCarthy[3].
[1]Department of Physiology, University College Cork, [2]The Robert Gordon University, Aberdeen, UK, and [3]The Irish Centre for Fetal and Neonatal Translational Research, Department of Obstetrics & Gynaecology, University College Cork, Ireland.

Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) is a transcriptional co-activator that drives mitochondrial biogenesis and other metabolic programs in many tissues, including the heart. PGC-1α is a powerful regulator of angiogenesis, a process of fundamental importance to the development of a healthy placenta in normal pregnancy. The angiogenic function of PGC-1α has been previously described in the heart where it has been shown to regulate angiogenesis. Its function and expression in the placenta is unknown. It may be the key link between placental development and the imbalance of key angiogenic factors observed in the pregnancy specific condition of pre-eclampsia. We hypothesized that PGC-1α is expressed in the human placenta and in pathophysiological conditions, such as pre-eclampsia, the expression of PGC-1α is reduced.

Human placental samples were obtained from normal healthy pregnant women for processing and analysed by means of immunohistochemistry using Calbiochem Anti PGC-1α, C-Terminal (777-797) rabbit polyclonal antibody. Rat cardiac (positive control) and placental samples from normal pregnant, sham pregnant and reduced uterine perfusion pressure (RUPP) rats were additionally obtained from a previous study by Walsh et al. and processed for immunohistochemistry with the human tissue.

Positive staining for PGC-1α was noted in both human and rat placental samples with varying degrees of expression when compared with PBS treated controls. Expression was most prominent in the endothelial cells surrounding the vessels, the trophoblasts, syncytiotrophoblasts and vascular endothelial cells with scattered staining in the maternal decidua and stroma. Furthermore, RUPP placental tissue indicated lower levels of positive staining for PGC-1α when compared with normal and sham rats.

This study indicates that the transcriptional co-activator PGC-1α is expressed in the placenta of both rat and human models of pregnancy. Furthermore, our data may implicate PGC-1α in the pathogenesis of complications of pregnancy such as pre-eclampsia, but further investigation is needed to confirm this. Future studies could suggest the use of PGC-1α as a future pharmacological target for the treatment of complicated pregnancies.

P27

NOVEL INTERACTIONS OF SUCCINATE, LIPOPOLYSACCHARIDE AND HYPOXIA ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS IN VITRO

L.M. Alvey, J.J. O'Connor.
UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

Succinate is an intermediate acid formed in the mitochondria during aerobic metabolism and in glial cells during the GABA/glutamine cycle. In physiologic conditions, its plasma concentration is in the micromolar range, but in times of physical stress, such as during intense exercise, inflammation and hypoxia, its concentration may increase to millimolar levels. Previous studies have found that succinate modulates synaptic transmission in the CA1 region of the hippocampus[1], while a very recent study reported that administration of exogenous succinate to macrophages increased the concentrations of pro inflammatory cytokines such as IL-1β[2]. Lipopolysaccharide (LPS) causes the release of pro inflammatory cytokines, which have previously been shown by our laboratory to attenuate the normal recovery of synaptic transmission following acute hypoxia. Therefore, we hypothesised that LPS would also attenuate recovery of synaptic transmission following acute hypoxia, and that the application of succinate may exacerbate this attenuation.

Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the medial perforant pathway in isolated slices of the dentate gyrus of the hippocampus. Long-term potentiation (LTP) was induced by high frequency stimulation with 3 trains separated by 20 s (each train of 1 s at 100Hz) in the presence of 100 μM picrotoxin. All data are presented as mean±sem and compared using the Student's t-test.

Application of 5 mM succinate significantly increased fEPSP slope (109.7±3.2%; n=8; $P<0.01$; compared to controls). We also demonstrated that LPS significantly attenuated the time to recovery of fEPSPs from acute hypoxia (80.0±5.9% hypoxia group; n=4; versus 64.9±2.4% LPS/Hypoxia group; n=4; $P<0.05$). We observed a bimodal effect of succinate on the fEPSP slope of slices co-treated with LPS and hypoxia. 50% of slices were unaffected whilst the other 50% surprisingly showed a significant improvement in recovery in the presence of succinate, during and after hypoxia.

Further work on the action of succinate on synaptic signalling and hypoxia is required to elucidate its role in neurodegenerative diseases such as stroke.

References

1. Roehrs, C. et al. (2004). Succinate increases neuronal post-synaptic excitatory potentials in vitro and induces convulsive behavior through N-methyl-d-aspartate-mediated mechanisms. *Neurosci.* 125(4), 965–71.
2. Tannahill, G.M. et al. (2013) Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature*. 496 (7444): 238-42.

P28

ACUTE HYPOXIC PRE-CONDITIONING AND PROLYL-HYDROXYLASE INIBITION IMPROVES SYNAPTIC TRANSMISSION RECOVERY TIME FROM A SUBSEQUENT HYPOXIC INSULT IN THE RAT HIPPOCAMPUS

A.E. Corcoran, J.J. O' Connor.
UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland.

Acute hypoxia is associated with numerous central nervous system disorders including stroke, Alzheimer's disease and obstructive sleep apnea. In the central nervous system it results in a decrease in synaptic transmission which may be fully reversible upon re-oxygenation. Stabilization of hypoxia-inducible factor (HIF) by inhibition of prolyl hydroxylase domain (PHD) enzymes has been shown to regulated the cellular response to hypoxia and confer neuroptrection *in vivo* and *in vitro*[1]. We have recently demonstrated a novel role for PHD inhibiton in acute hypoxia and synaptic transmission and plasticity[2]. Hypoxic preconditioning has become a novel therapeutic target to induce neuroprotection during hypoxic insults. However, the effects of repeated hypoxic insults or pharmacological PHD inhibition on synaptic signalling remain unknown. Therefore we aimed to assess the effects of hypoxic preconditioning and PHD inhibition on the acute response to hypoxia in the rat hippocampus.

Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the Schaffer collatoral pathway in isolated slices of the CA1 region. Long-term potentiation (LTP) was induced by high frequency stimulation with 3 trains separated by 20 s (each train of 1 s at 100Hz). 30 min hypoxia resulted in a significant and reversible decrease in fEPSP amplitude associated with decreases in partial pressures of oxygen. 15-30 min of hypoxia was sufficient to induce stabilization of HIF in hippocampal slices. Exposure to a second hypoxic insult after 60 min resulted in a similar depression of fEPSP slope. However re-oxygenation of hippocampal slices following the second exposure to hypoxia resulted in a significantly greater rate of recovery of fEPSP. 60 min treatment of slices with the PHD inhibitor, dimethyloxalyl glycine (DMOG, 1 mM) caused a small but significant decrease in fEPSP slope after 30 minutes. Re-oxygenation, following 30 min acute hypoxia in DMOG treated slices, also resulted in a significantly greater rate of recovery of fEPSP slope compared to controls.

These results suggest that hypoxia and 'pseudohypoxia' preconditioning may improve the rate of recovery of hippocampal neurons to acute hypoxia.

References

1. Batti, L., Taylor, C.T., O'Connor J.J. (2010). Hydroxylase inhibition reduces synaptic transmission and protects against a glutamate-induced ischemia in the CA1 region of the hippocampus. *Neurosci,* 167, 1014-1024.
2. Corcoran, A., Kunze, R., Harney, S.C., Breier, G., Marti, H.H., O'Connor, J.J. (2013) A role for prolyl hydroxylase domain proteins in hippocampal synaptic plasticity. *Hippocampus, in press.*

P29

A NOVEL ROLE FOR ADRENERGIC AND SEROTONERGIC SIGNALING IN THE ACTION OF ERGOMETRINE IN ISOLATED TERM HUMAN MYOMETRIUM

C. Leyden[1], R.A. Fanning[2], D.P. Campion[3], J.J. O'Connor[1].
[1]UCD School of Biomolecular & Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, [2]Department of Perioperative Medicine, Coombe Women and Infant's University Hospital, Dublin, and [3]UCD School of Veterinary Medicine, University College Dublin, Ireland.

Ergometrine is an ergot alkaloid used clinically in the management of postpartum hemorrhage (PPH). It acts rapidly by inducing forceful contraction of the myometrial smooth muscle to halt excessive blood loss. Despite its long-standing use in the management of PPH, the mechanism by which it acts in the human uterus is still unknown. Previous research into ergometrine's action as an oxytocic in rat myometrial tissue has indicated that its mechanism of action is likely to be via 5-HT signaling[1]. However the adrenergic system has also been implicated to have a role in its mechanism of action in human myometrial tissue[2].

Using samples of pregnant human myometrial tissue obtained from the Coombe Women and Infant's University Hospital, we were able to verify the uterotonic actions of ergometrine on spontaneous isolated human myometrial contractions, versus time-matched controls. All patients gave written consent to donation of the myometrium tissue and the protocol was approved by the Institutional Review Board of the Hospital. This study used four antagonists targeted at receptors involved in 5-HT and adrenergic signaling to investigate their role in the effect of ergometrine on human myometrial tissue.

Metergoline, a non-specific 5-HT antagonist inhibited the ergometrine induced increase in amplitude of contractions ($p<0.05$). RS-127445, a specific 5-HT_{2B} receptor antagonist, but not ketanserin (5-HT_{2A} antagonist), also inhibited the effect of ergometrine on amplitude of contractions ($p<0.05$). Phentolamine, an α-adrenergic receptor antagonist also inhibited the increase in amplitude of contractions following administration of ergometrine ($p<0.05$). Propranolol, a non-specific β adrenergic receptor antagonist had no significant effect on the actions of ergometrine.

These results provide novel evidence that both adrenergic (α) and serotonergic (5-HT_{2B}) signaling mechanisms may be involved in the mechanism of action of ergometrine in human myometrial smooth muscle.

References

1. Hollingsworth M, Edwards D, Miller M. (1988) Ergometrine--a partial agonist at 5-HT receptors in the uterus isolated from the oestrogen-primed rat. Eur J Pharmacol. 158: 79-84.
2. Dyer RA, van Dyk D, Dresner A. (2010) The use of uterotonic drugs during caesarean section. Int J Obstet Anesth. 19: 313-9.

P30

EFFECTS OF CHRONIC INTERMITTENT HYPOXIA AND PROLYL-HYDROXYLASE INHIBITION ON SYNAPTIC TRANSMISSION AND PLASTICITY IN THE RAT CA1 AND DENTATE GYRUS

A. Wall[1], A.E. Corcoran[1], K.D. O'Halloran[2], J.J. O' Connor[1].
[1]UCD School of Biomolecular and Biomedical Science, Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Ireland and
[2]Department of Physiology, School of Medicine, University College Cork, Ireland.

Chronic intermittent hypoxia (CIH) is a dominant feature of obstructive sleep apnoea and has been shown to have deleterious and damaging effects on neurons and to impair synaptic plasticity in the CA1 region of the rat hippocampus[1]. CIH is a potent inducer of hypoxia inducible factors (HIF), which is a key regulator in cellular adaptation to hypoxia that plays an important role in the fate of neurons during ischemia. Levels of HIF-1α are regulated by the activity of a group of enzymes called HIF-prolyl 4-hydroxylases (PHDs) and these have become potential pharmacological targets for preconditioning against ischemia. However, little is known about the effects of CIH and prolyl hydroxylase inhibition on synaptic transmission and plasticity in sub-regions of the hippocampus.

Male Wistar rats were treated for 7 days with either CIH (20 cycles per hour for 8h per day), dimethyloxalyl glycine (DMOG), 50 mg/kg i.p. every 3 days, or saline i.p.. At the end of the treatments, animals were anaesthetised with 5% isoflurane by inhalation and decapitated. 350 µm transverse hippocampal slices were prepared. Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation in the Schaffer collatoral (CA1) or medial perforant path (dentate gyrus) of the hippocampus using a monopolar glass electrode. Long-term potentiation (LTP) was induced by high frequency stimulation with 3 trains of 100 Hz stimuli separated by 20 s. The effect of acute hypoxic conditions (delivery of 95%N_2/5%O_2 for 30 min) on the fEPSP slope was measured every 30 s. All data are presented as mean±SEM and compared using the Student's t-test.

At the end of the treatment periods, all 3 groups showed no change in synaptic excitability or paired pulse paradigms. LTP was impaired in the CA1 region of the hippocampus in both CIH and DMOG treated animals compared to shams (111.1±10.6%, n=5, **P<0.01 and 113.4±7.1%, n=7, **P<0.01 vs 150.4± 5.5%, n=8). LTP, induced in the dentate gyrus was of similar magnitude in all groups (Sham, 118.4±1.7%, n=5 vs CIH, 114.9±4.6%, n=5 vs DMOG, 125.2±7.2%, n=4). We also investigated the effect of 7-day CIH and DMOG treatment on the recovery of synaptic transmission from a 30 min hypoxic exposure. CIH treated animals showed a more rapid recovery of fEPSP slope post hypoxia in both the CA1 and dentate gyrus regions. This effect was not seen with 7-day DMOG treatment.

These results suggest that LTP induction in the CA1 region is more sensitive to both CIH and DMOG treatment than the dentate gyrus. However CIH treatment alone alters the sensitivity of hippocampal neurons to acute hypoxia.

Reference

1. Xie H, Yung W. (2012). Chronic intermittent hypoxia-induced deficits in synaptic plasticity and neurocognitive functions: a role for brain-derived neurotrophic factor. Acta pharmacologica Sinica. 33: 5–10.

P31

XANTHINE OXIDASE INHIBITION IMPROVES RAT PHARYNGEAL DILATOR MUSCLE FUNCTION IN VITRO

D. Burns, P. Lewis, K.D. O'Halloran
Department of Physiology, School of Medicine, University College Cork, Ireland.

The use of antioxidants as muscle inotropes and therapeutic agents is increasing. However, there is paucity of information concerning their effects on isotonic muscle function. Xanthine oxidase is a ROS generating enzyme ($O_2\bullet^-$ and H_2O_2) implicated in muscle and inflammatory disorders. We sought to determine if pharmacological treatment *in vitro* using the xanthine oxidase inhibitor Oxypurinol, alters the contractility of the sternohyoid muscle - a representative pharyngeal dilator implicated in the control of airway patency.

Sternohyoid muscle was excised from 16 young adult male Wistar rats (200-350g) following humane euthanasia under 5% isoflurane anaesthesia and mounted *in vitro* between platinum electrodes in Krebs solution. Muscle was stimulated under hyperoxic conditions (95% O_2, 5% CO_2) at 35^{0}C, in the presence (n=8) or absence (control, n=8) of 1mM Oxypurinol. Using a dual-mode lever system, peak twitch and tetanic force were determined under isometric conditions. Under isotonic conditions, muscle shortening and velocity of shortening was determined at varying loads (0-100%). Muscle work, power and isotonic fatigue were determined.

Oxypurinol treatment resulted in significantly increased peak twitch (2.6 ± 0.3 *versus* 1.8 ± 0.1N/cm^2; Oxypurinol versus control; p=0.0378, Student's unpaired t-test) and tetanic force (19.0 ± 1.9 *versus* 10.4 ± 0.6 N/cm^2; p=0.0024) compared to control conditions. Peak mechanical work was higher in the presence of Oxypurinol compared to control values (1.2 ± 0.3 versus 0.5 ± 0.1 J/cm^2; p=0.0399). Oxypurinol treatment resulted in significantly increased mechanical work (p<0.0001, two-way ANOVA) and power (p=0.0046) production across the entire load continuum (0 to 100% load). During repeated stimulation at 20% load, work and power were significantly increased by oxypurinol, but only in the early portion of the test. All values mean ± SEM.

Oxypurinol had a positive inotropic effect on rat sternohyoid muscle and resulted in significantly increased work and power production across the entire load continuum. The data suggest that ROS from cytosolic oxidases are important regulators of respiratory muscle function. These results are important in the context of upper airway muscle impairment such as that reported in obstructive sleep apnoea syndrome. Antioxidants may be a realistic therapeutic option for respiratory disorders characterised by respiratory muscle weakness and fatigue.

Supported by the Department of Physiology, University College Cork, Ireland.

P32

AN INVESTIGATION OF THE EFFECT OF GREEN TEA EXTRACT (CAMELLIA SINENSIS) SUPPLEMENTATION ON FAT METABOLISM IN EXERCISING HUMANS

M. Corcoran[1], J. Bradley[2], T. Woods[3], K.D. O'Halloran[1].
Departments of [1]Physiology, [2]Education and [3]Sport and Physical Activity, University College Cork, Cork, Ireland.

It is suggested that Green Tea Extract (GTE) may potentiate fat oxidation during exercise (1). Such interventions may have clinical relevance as adjunct

treatments in metabolic diseases such as obesity or type 2 diabetes (2). The aim of this study was to investigate if a pre-exercise, orally administered, GTE supplement enhances fat oxidation in exercising young healthy humans compared to a placebo.

Ten subjects (5 male) were recruited (age 20-23; male weight range 56-95kg; female weight range 50-61kg; BMI range 18.9 – 27.6 kg/m^2). Ethical permission was granted by The Clinical Research Ethics Committee, UCC and all subjects gave informed consent. Subjects were required to attend the laboratory for 3 tests, each lasting 1-3 hours. The first test was used to determine the subjects' respiratory exchange ratio during incremental exercise on a stationary bicycle ergometer (30 watt increments every 5 minutes up to a max of 150 watts). Peak fat oxidation rate was calculated and the associated exercise intensity was used for the subsequent tests. During tests 2 and 3, subjects first consumed either GTE or placebo, in a single blind, crossover design and after 45min performed a 20 minute bout of exercise at the 'peak fat oxidation' intensity determined during test 1. The average fat oxidation (g/min) during the exercise bout was calculated off-line.

GTE supplementation significantly increased fat oxidation during the 20 minute exercise bout compared to placebo by ~30% (0.227 ±0.039g/min vs. 0.174 ±0.038g/min; GTE vs. placebo; P=0.0085 Student's paired t-test).

GTE supplementation may modulate metabolic rate in health and disease (e.g. obesity). GTE also has the potential to be an aid in endurance events, where it is advantageous to use fat stores thereby limiting the use of muscle glycogen. It is possible that GTE or catechins could be used as a legal performance enhancer in endurance sports. Further studies on the effects of GTE in obese patients and elite athletes are warranted.

References

1. Jeukendrup AE, Wallis GA. (2005) Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* 26:S28–37.
2. Thavanesan N. (2011) The putative effects of green tea on body fat: an evaluation of the evidence and a review of the potential mechanisms. *Br. J. Nutr.* 106, 1297–1309.

P33

EFFECTS OF NEONATAL EXPOSURE TO CHRONIC INTERMITTENT HYPOXIA ON RAT RESPIRATORY MUSCLE FUNCTION AND REDOX STATUS

R. Williams[1], F. McDonald[3], V. Healy[1], D. Sheehan[2] and K.D. O'Halloran[1]
Departments of [1]Physiology and [2]Biochemistry, University College Cork, and
[3]School of Medicine and Medical Science, Health Sciences Centre, University College Dublin, Ireland.

Chronic intermittent hypoxia (CIH) is common in disorders of respiratory control, such as apnoea of prematurity, due to recurrent hypoventilation and respiratory cessation. There is a growing body of evidence to suggest that CIH causes aberrant remodelling at multiple sites of the respiratory control system. Early life represents a particularly vulnerable period for maladaptive plasticity in homeostatic control systems and the striated muscles of breathing undergo

considerable change in form and function during neonatal development. The aim of this study was to investigate the effects of CIH exposure during neonatal development on rat sternohyoid (SH) and diaphragm (Dia) muscle. We hypothesized that CIH would cause respiratory muscle dysfunction secondary to oxidative stress.

Neonatal male and female Wistar rats were exposed to alternating cycles of 90s hypoxia (5% O_2 at nadir) and 210s of normoxia (i.e. 21% O_2, 12 cycles per hour), for 8 hours a day, for 21 consecutive days from the first day of life. Control (sham) rats were exposed to normoxia in parallel. The SH and Dia muscles were excised post-mortem (humane euthanasia performed under 5% isoflurane anaesthesia) and muscle bundles were suspended in custom tissue baths containing Krebs solution at 35°C, bubbled with 95% O_2 and 5% CO_2 for control experiments and 95% N_2 and 5% CO_2 for hypoxic bath experiments for comprehensive assessment of contractile and endurance properties. In addition, muscle samples were processed for determination of free thiol and carbonyl group content using fluorescent tags which were separated using 1D electrophoresis, imaged using a fluorescence scanner and analysed using densitometry. Data were statistically compared using Student's *t*-tests and two-way ANOVA as appropriate; significance was taken at $P<0.05$.

Functional analysis of peak isometric force and force-frequency relationship revealed that CIH caused significant SH muscle weakness in both male ($P<0.05$) and female ($P<0.05$) rats with no significant change in muscle endurance. There was no significant effect on Dia function. Biochemical analysis provided no evidence of an accompanying oxidative stress in CIH-treated respiratory muscles i.e. no significant change in free thiol and carbonyl group content. Interestingly, muscle- and sex-specific differences were noted.

Our study demonstrates that CIH exposure during neonatal development causes sternohyoid muscle weakness. Contrary to expectation, the underlying mechanism may be unrelated to overt free-radical mediated dysfunction. The sternohyoid is one of a group of muscles critical in the control of upper airway calibre. Dysfunction increases the propensity for airway collapse, which if it occurred in humans could serve to exacerbate sleep-disordered breathing. Further investigation is required to determine the mechanism(s) contributing to CIH-induced respiratory muscle dysfunction.

Funded by the Health Research Board (Ireland) and the Department of Physiology, University College Cork, Ireland (RW).

P34

CHRONIC INTERMITTENT HYPOXIA INCREASES NADPH OXIDASE SUBUNIT EXPRESSION IN RAT STERNOHYOID MUSCLE

R. Williams[1], E. Lucking[3], D. Sheehan[2], V. Healy[1], K.D. O'Halloran[1]
Departments of Physiology[1] and Biochemistry[2], University College Cork, Ireland and [3]School of Medicine and Medical Science, Health Sciences Centre, University College Dublin, Dublin, Ireland.

Chronic intermittent hypoxia (CIH), a defining feature of obstructive sleep apnoea (OSA), is recognised as a key stimulus driving aberrant respiratory muscle remodelling. OSA affects at least 2-4% of the adult population and is recognised as a significant public health issue[1]. We have shown that exogenous antioxidant

supplementation ameliorates CIH-induced muscle weakness[2], implicating reactive oxygen species (ROS) in CIH-induced muscle impairment. The superoxide generating enzyme - NADPH oxidase (NOX), is implicated in CIH-induced plasticity in sensory and motor nerves. The aim of this study was to determine the effects of CIH on NOX subunit expression and markers of oxidative stress in rat sternohyoid muscle (SH), an upper airway dilator muscle important in the control of airway patency.

Adult male Wistar rats were exposed to alternating cycles of 90s hypoxia (5% O_2 at nadir) and 210s of normoxia (21% O_2) i.e. 12 cycles per hour, for 8 hours a day, for 2 weeks. Control (sham) rats were exposed to normoxia in parallel. SH muscles were excised post-mortem (humane euthanasia was performed under 5% isoflurane anaesthesia) and processed for further analysis using western blotting to determine NOX2, p47 phox and p22 phox subunit expression. Fluorescent tagging of free thiol and carbonyl groups was conducted to determine redox status of the muscle. Following densitometric analysis, data were compared using Student's *t*-tests; significance was taken at $P<0.05$.

We observed a near three-fold increase in NOX2 expression ($P<0.05$), a near two-fold increase in p47 phox expression ($P=0.0143$) and no alteration in p22 phox expression in the SH of CIH-treated rats compared to sham. However, fluorescent tagging of free thiol and carbonyl groups revealed no significant alteration in redox state of the CIH-treated SH muscle compared to control.

Our study demonstrates a CIH-induced increase in the expression of key NADPH oxidase subunits. Despite this finding, we did not observe any appreciable alteration in the redox state of the SH muscle following CIH treatment. We will next establish if NOX activity is increased following CIH exposure. Our results suggest that CIH-induced respiratory muscle dysfunction is not a result of overt oxidative stress, but may be related to an increase in ROS signalling perhaps through cytosolic oxidases such as NOX. Of interest, preliminary studies from our group indicate that NOX inhibition with apocynin increases sternohyoid muscle force *in vitro*.

Funded by the Health Research Board (Ireland) and the Department of Physiology, University College Cork, Ireland (RW).

References

1. Young T., Finn L., Peppard P.E., Szklo-Coxe M., Austin D., Nieto F.J., Stubbs R., Hla K.M. Sleep disordered breathing & mortality: eighteen-year follow-up of the Wisconsin sleep cohort. Sleep 2008; 31: 1071-8.
2. Skelly J.R., Edge D., Shortt C.M., Jones J.F.X., Bradford A., O'Halloran K.D. Tempol ameliorates pharyngeal dilator muscle dysfunction in a rodent model of chronic intermittent hypoxia. American Journal of Respiratory Cell and Molecular Biology 2012; 46: 139-48.

P35

DIAPHRAGM MUSCLE REMODELLING IN A RAT MODEL OF CHRONIC INTERMITTENT HYPOXIA

C. Shortt[1], A. Fredsted[2], A. Bradford[3], J.F.X. Jones[1], K.D. O'Halloran[4].
[1]UCD School of Medicine and Medical Science, University College Dublin, Ireland, [2]Department of Biomedicine, Aarhus University, Denmark, [3]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, and [4]Department of Physiology, School of Medicine, University College Cork, Ireland.

P36

NEONATAL EXPOSURE TO CHRONIC INTERMITTENT HYPOXIA CAUSES PERSISTENT RESPIRATORY MUSCLE WEAKNESS IN MALE AND FEMALE RATS

F.B. McDonald[1], D. Edge[1], K.D. O'Halloran[2].
[1]UCD School of Medicine and Medical Science, University College Dublin and [2]Department of Physiology, School of Medicine, University College Cork, Ireland.

P37

EFFECT OF CHRONIC INTERMITTENT HYPOXIA ON THE REFLEX RECRUITMENT OF THE GENIOGLOSSUS DURING AIRWAY OBSTRUCTION IN THE ANAESTHETISED RAT

D. Edge[1], F.B. McDonald[1], J.F.X. Jones[1], A. Bradford[2], K.D. O'Halloran[3].
[1]UCD School of Medicine and Medical Science, University College Dublin, [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, and [3]Department of Physiology, School of Medicine, University College Cork, Ireland.

P38

THE EFFECTS OF VARIATIONS IN THE N-TERMINUS ON THE SENSITIVITY OF CHICK TRPM8 TO MENTHOL AND COLD

N. Morshed, G. Reid
Department of Physiology, University College Cork, Ireland.

The cold and menthol receptor TRPM8 is the principal detector of cooling in the skin and a potential drug target in cold allodynia and prostate cancer. The mechanism and structures that underlie menthol and cold sensitivity in TRPM8 are not fully understood. This study addresses this question using naturally occurring variation, with two variants of TRPM8 cloned from *Gallus gallus domesticus* using primers designed from *Gallus gallus*. They exhibited different responses to cold in whole cell patch clamp recordings: type 1 displayed a cold-induced current activating at around 37 °C while type 2 did not respond to cold, but did respond to simultaneous cold and menthol stimulation.

The type 1 and type 2 variants differ at only four sites. These are a 7 amino acid (406-412) insertion/deletion and three single amino acid changes. These are located in the n-terminus of the protein, an area whose role is not fully understood. Chimeras were constructed, based on the type 1 clone, with each containing one of the differences.

The TRPM8 variants and chimeras were transiently expressed in HEK293t cells. Responses to a series of cold (ramp from 32 °C to 8 °C) and menthol (100 μM) stimuli were measured using Calcium Green-1 microfluorimetry ($\Delta F/F_0$). Type 1 responded to cold with an increase in intracellular [Ca^{2+}], with $\Delta F/F_0$ = 0.511 (median), 0.361-0.648 (interquartile range), significantly greater than in

untransfected cells (0.0792(0.0313-0.241), p<0.001, Mann-Whitney rank sum test). In contrast, cooling alone did not elicit a significant response in Type 2 (0.172(0.0617-0.259), p=0.267). When menthol (100 μM) and cooling were applied simultaneously, Type 1 (0.646(0.528-0.800)) and type 2 (0.640(0.525-0.841)) responses were not significantly different (p=0.690). Only one chimera, containing the 406-412 amino acid deletion, was found to have a smaller (0.118(0.0583-0.240)) response to menthol than type 1 (p<0.001). The response was similar to that in untransfected HEK293t cells (p=0.962), while still larger than the type 2 response (p=0.038)

Levels of expression of the two variants were examined in native DRG using qRT-PCR. These results show that, while type 1 and type 2 respond differently to cold and menthol stimuli, no single difference in their sequences is responsible for the differing responses.

Financially supported by the Department of Physiology, University College Cork.

P39

REDUCED 15-LO2 AND LIPOXIN A_4 / LEUKOTRIENE B_4 RATIO IN LOWER AIRWAYS OF CHILDREN WITH CYSTIC FIBROSIS

F.C Ringholz, P. McNally, V. Urbach.
National Children's Research Centre, Crumlin, Dublin, Ireland.

P40

DISCOVERY OF CADHERIN-5 PROTEIN IN HUMAN PLATELETS

Golla. K[1] .,Treumann, A[3] ., Shields. D[2]., Moran. N[1]

[1]Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, [2]Complex and Adaptative systems Laboratory (CASL), University College Dublin, Ireland, and [3]Biopharmaceutical Bioprocessing Technology Centre, Newcastle University, UK.

Previous work in our laboratory has identified novel short cell-permeable peptide modulators of platelet secretion derived from cadherin cell adhesion molecules[1]. Peptides were identified based on patterns of evolutionary conservation. Here we further explore the nature of cadherins and cadherin-associated proteins in human platelets.

Washed platelets were prepared from blood obtained from healthy donors. Platelet aggregation was performed in a PAP-8 aggregometer. Platelets lysates were prepared after aggregation and analysed for expression of cadherin-1, cadherin-2, cadherin-5 and cadherin-6 using western blotting. P120 catenin, beta catenin, alpha catenin and Junctional Plakoglobin antibodies were used for detection of cadherin-associated proteins.

Using western blotting techniques, we have identified Cadherin-5 and Cadherin-6 but not Cadherin-1 or Cadherin-2 in human platelets. In addition, we identified cadherin-associated protein such as P120 catenin. To confirm the specificity of the antibodies used in our studies, immunoprecipitaion experiments were performed and relevant bands were excised from SDS gels, trypsin-digested and samples were analysed in mass spectrometry to determine the nature of the cadherin present. Cadherin-5 but not cadherin- 6 was identified in immunoprecipitates of cadherin-5 antibody. Conversely, cadherin-6 but not cadherin-5 was obtained from cadherin-6 antibody precipitates, confirming the specificity of the antibodies used. Cadherin-5 expression was more abundant

compared to Cadherin-6. Cadherin-5 expression is not altered during platelet aggregation events. Cadherin 5 is not phosphorylated in the course of platelet aggregation. Cadherin-5 blocking antibody did not inhibit platelet aggregation response. Thus the role of cadherin-5 in platelets remains unclear.

This study identified novel cadherin-5 and its associated protein p120 catenin in platelets. However, the function of this cadherin in platelets remains elusive.

Reference

1. Edwards, RJ., Moran, N., Devocelle, M., Kiernan, A., Meade, G., Signac, W., Foy, M., Park, S., Dunne, E., Kenny, D.& Shields, D. Bioinformatic discovery of oligopeptides. Nature Chem Biology 3, 108-112 (2007).